CRANFIELD UNIVERSITY

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UNDERSTANDING THE CHANGES IN SRI LANKAN MANGO FRUITS DURING POSTHARVEST RIPENING

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UNDERSTANDING THE CHANGES IN SRI LANKAN MANGO FRUITS DURING POSTHARVEST RIPENING

Supervisor: Dr. Leon A. Terry

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for the degree of Doctor of Philosophy

ABSTRACT

This is the first study to be carried out which describes changes in biochemical profile of both pre- and post-climacteric Sri Lankan mango fruits. Chemometric analysis revealed that spatial distribution of biochemical compounds between peel and pulp was the major discriminatory factor during maturation; whereas fully mature mango fruit contained the highest concentration of starch and lowest concentration of acids. Since these combined variables also responsible for the final quality of ripe fruit, they could be used as important parameters of horticultural maturity to inform growers to harvest Sri Lankan mango fruit at the optimum stage.

In further work, mango fruits were ripened at 32^{o}C for 4 days in order to better understand the temporal variation of major biochemical components during postharvest ripening. Mango cv. Malgova contained about three-fold higher organic acids than other cultivars tested, whilst cv. Willard had significantly higher glucose and ascorbic acid levels than cvs. Karutha Colomban and Malgova. In addition to differences in sugars and acids, mango peel had about ten-fold higher total phenolics than pulp, whereas cv. Willard pulp contained comparatively higher total phenolics than cv. Malgova. Therefore, many consumers may prefer both cvs. Willard and Karutha Colomban as being of superior flavour and quality as compared to cv. Malgova since they had comparatively higher sugar/acid ratio, antioxidant capacity and a vibrant yellow-orange colour when ripe. Malgova fruits are less suitable as a dessert mango, but are better suited to be used in pickles. Mangiferin (most abundant flavonoid in the cultivars tested) has a variety of reported health-related properties (antioxidant, antitumor and antiviral) and thus differences in biochemical composition could be used to promote sales. Ascorbic acid, and some carotenoids and phenolic compounds are also antioxidants.

In order to elucidate the temporal effects of ripening temperature on quality-related target analytes, mango fruits were either ripened at 20°C or 30°C (or a combination of the two). The aim was to identify the best ripening temperature and then potentially advise practitioners to use appropriate temperature in order to attain superior fruit quality. The sugar/acid ratio and total carotenoids increased more in fruits ripened at 30°C than 20°C whilst total phenolics and flavonoids increased in fruits ripened at 20°C, but decreased in fruits ripened at 30°C. Ripening at 20°C enhanced the overall biochemical profile, quality parameters and shelf-life of mango fruit. As a consequence, the implications of this research are that Sri Lankan mango fruit should be ripened at a lower temperature, such that current practices should change.

The genotypic composition of volatiles in Sri Lankan mango fruit and the effect of postharvest storage on these volatiles has never been attempted. Using a new extraction and quantification methodology developed as part of this research, the profile of volatiles varied significantly amongst the cultivars studied. Whereas terpinolene was most abundant in cv. Willard, ocimene and myrcene were the prominent compounds measured in cvs. Karutha Colomban and Malgova, respectively. Terpinolene possess a floral, sweet and pine-like aroma, whilst ocimene and myrcene are responsible for the often quoted 'green' aroma of some mango fruit. Given these differences it would, therefore, be expected that cv. Willard fruits would be adjudged favourably by more consumers and perhaps be better suited for export.

It is evident from the work presented herein that when comparing the biochemical profiles of cvs. Willard and Karutha Colomban to most mainstream commercial cultivars currently available on the global market that these endemic cultivars may be preferred by more consumers.

ACKNOWLEDGEMENTS

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LIST OF CONTENTS

LIST OF FIGURES

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

CHAPTER SIX

LIST OF TABLES

CHAPTER TWO

CHAPTER THREE

CHAPTER FOUR

NOTATION

<	less than
>	greater than
±	more or less equal
%	per cent
=	equals
°C	degree Celsius
β	beta
α	gamma
µl	microlitre
µg	microgram
1-MCP	1-methycyclopropene
ACC	1-aminocyclopropane-1-carboxilic acid
AACC	American Association of Cereal Chemists
ACCO	1-aminocyclopropane-1-carboxilic acid oxidase
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemists

AsA	ascorbic acid
CA	controlled atmosphere
ca.	approximately
Ca	calcium
$CaCl_2$	calcium chloride
CIMS	chemical ionization mass spectrometry
cm	centimetre
CO_2	carbon dioxide
cv.	cultivar
cvs.	Cultivars
DAFB	day after full bloom
BAFS	day after fruit set
d.f.	degrees of freedom
DW	dry weight
EEC	European Economic Community
EIMS	electron ionization mass spectrometry
ELSD	Evaporative Light Scattering Dectector
et al.	and others
FAO	Food and Agriculture Organisation of the United Nations
FID	Flame Ionisation Detection
FW	fresh weight
g	gram
GAE	gallic acid equivalent
GC	Gas Chromatography

h	hours
ha	hectare
HCA	hierarchical component analysis
HCl	hydrochloric acid
HPLC	High Performance Liquid Chromatography
ICC	International Association for Cereal Science and Technology
in vitro	outside a living organism
in vivo	inside a living organism
kg	kilogram
kN	kiloNewton
kPa	kiloPascal
l	litre
LDPE	low density polyethylene
LSD	least significant difference
M	molarity
m	metre
MA	modified atmosphere
mg	milligram
min	minute
ml	millilitre
mm	millimetre
mM	millimolar
MW	molecular weight
N	normality

N_2	nitrogen
NA	not applicable
NM	not measured
NaCl	sodium chloride
NaOH	sodium hydroxide
ng	nanogram
$(NH_4)_2SO_4$	ammonium sulphate
NSC	non-structural carbohydrate
nm	nanometre
nmol	nanomole
OA	organic acids
O_2	oxygen
P	probabiltity
PCA	principal component analysis
RH	relative humidity
rpm	revolutions per minute
RR	respiration rate
SDW	sterile distilled water
S.E.	standard error
SIMCA	soft independent modelling of class analogy
TLC	thin layer chromatography
TP	total phenolics
TSS	total soluble solids
TTA	total titratable acidity

UK	United Kingdom
US	United States
USA	United States of America
USDA	United States Department of Agriculture
viz.	namely
v/v	volume by volume
w/w	weight by weight

CHAPTER ONE

Introduction

1.1 Background

Mango (*Mangifera indica* L.) (King of fruits) belongs to the genus *Mangifera*, consisting of numerous species of tropical fruit trees in the family Anacardiaceae. Mango is considered indigenous to Eastern Asia, Myanmar and the Assam state of India, however mango is now grown extensively as a fruit tree in most tropical and sub-tropical regions (Morton, 1987). Ripe mangoes are being consumed mostly as a dessert fruit, but mature mangoes are also eaten fresh in pickles, sliced or grated in fresh salad, soaked in water or sugar, salted and dried and sliced in vinegar or fish sauce. Mango has special importance in Pakistan, India, Bangladesh, Sri Lanka and Philippines as its leaves are spiritually used in religious ceremonies (Anon, 2009b; Kim *et al*., 2009). Total global mango production is around 28.51 million tonnes per annum (FAO, 2007), however the global production of mango is forecast to reach 30.7 million tonnes by 2010 (FAO, 2003).

Mango is an important horticultural crop cultivated in the main agro-ecological regions of Sri Lanka *viz.* dry, wet and intermediate zones. Total mango production in Sri Lanka is around 96,500 tonnes per annum. Mangoes are grown on 26,000 ha of land and derived from the three different agro–ecological regions (Peiris and Senevirathna, 2001). Unique colour, flavour, sweetness and aroma profiles are distinct properties of mangoes harvested in the dry zone, which attain greater consumer demand. However, lower production volumes along with high pre- and postharvest losses limit processing and export possibilities. A seasonal mango harvesting pattern is observed in Sri Lanka

as crops bloom from January to March and are harvested in May to July in wet and intermediate zones (Yala season crops). Mangoes from the dry zone bloom between July to September and are harvested November to January (Maha season crops). Therefore, production drops in between the two seasons, resulting in higher prices (Peiris and Senevirathna, 2001). Much research has been undertaken globally to quantify non-structural carbohydrates (NSCs), organic acids and total phenolics (TP) in various mango cultivars (Appendix C). However, published information on the biochemical profile of mangoes which are endemic to Sri Lanka is very limited to non-existence.

Mangoes should be harvested at fully mature stage for subsequent postharvest ripening. However, harvesting criteria vary according to local consumption patterns and distance to market. Fruit maturity at harvest is commonly judged by the shape of fruits, however days after flower set (DAFS) is one of the most critical factors affecting subsequent ripening, flavour development and postharvest utilisation (Lizada, 1993). According to Kosiyachinda *et al*. (1984), external properties *viz.* number of days after full bloom (DAFB) or DAFS, protrusion of shoulder, peel colour and a degree of 'bloom' on the fruit surface have also been used as predictors of harvest maturity. However, the eating quality is not affected by these said properties. Fully mature mangoes gain a better eating quality once ripe whilst immature ones do not; thus measuring the harvest quality of pre-climacteric mango is important in order to discriminate immature and mature fruits at harvest (Saranwong *et al*., 2004). The concentration of accumulated starch and dry matter are used to determine harvest quality (Tandon and Kalra, 1983; Ueda *et al*., 2000). Starch is the major carbohydrate present in mature mangoes and is hydrolysed into sugars during ripening (Lima *et al*.,

2001). Only trace amounts of starch and reduced amylase activity can be detected in over-ripe mangoes (Lima *et al*., 2001).

Generally, climacteric fruits like mango undergo biochemical changes during postharvest ripening *viz*. increased respiration, ethylene production, carbohydrate depolymerisation, organic acid degradation, softening, chlorophyll degradation and biosynthesis of carotenoids and aroma compounds, etc. (Lalel *et al*., 2003a; Vasquez-Caicedo *et al*., 2004; Rathore *et al*., 2007). Mango fruit is well known for its characteristic aroma and sweetness. In addition, mango fruit is also an excellent overall nutritional source of carbohydrates and dietary antioxidants (Kauer and Kapoor, 2001) such as ascorbic acid, carotenoids (*β*-carotene), phenolic compounds, vitamin E (α-tocopherol) and minerals. Dietary antioxidants also have reported immunomodulatory activity (Naved *et al*., 2005), antimutagenic properties (Botting *et al*., 1999) and anticancer activity (Percival *et al*., 2006).

1.2 Aim and objectives

1.2.1 Rationale

Mango production in Sri Lanka is seasonal, meaning that excess production during Maha and Yala seasons leads to lower prices in the domestic market whilst shortage of mango during off season increases the market price. Lack of proper storage, processing and marketing facilities, and no export possibilities are major causes for drop in price during mango seasons. Harvest maturity is one of the important parameters that determines the quality and nutritional composition of mango fruit. Mango should be harvested at appropriate maturity to develop favourable colour, flavour and nutritional

properties during subsequent postharvest ripening. Harvest maturity of mango fruit is determined using some physical and visual observations; however determining the harvest maturity of pre-climacteric mangoes using chemical profiles is considered as a more effective and objective method. Published information on biochemical properties of Sri Lankan mango cultivars are very limited, therefore analyzing biochemical compounds which are prominent in Sri Lankan mango cultivars may improve export possibilities.

Average temperature in Sri Lanka is ± 30^{o}C and the preferred temperature for ambient storage transport is around 20^{o}C. Therefore, studies on postharvest mango fruits to extend their shelf-life and analysing spatial and temporal variations of biochemical compounds using sophisticated instruments would be useful to suggest alternative optimum storage conditions. There are no reliable methods to extract and analyse aroma compounds from mango fruits, therefore it was necessary to develop a new method to extract and quantify prominent aroma compounds of Sri Lankan mango fruits using combined GC-FID and HS-SPME techniques within relatively a short time and at low temperature. In addition, there was a necessity to improve existing extraction and quantifying methods of flavonoids and total carotenoids from mango fruits in order to optimise extraction efficiency.

Chemometric analysis is an important approach to interpret mass data into meaningful conclusions. Principal Component Analysis (PCA) and Hierarchical Component Analysis (HCA) are unsupervised techniques and thus can be used to interpret the data without bias towards a desired outcome.

1.2.2 Hypotheses

- Sri Lankan mango fruits have higher concentration of starch at fully mature stage than other fruit development stages
- Biochemical compounds responsible for taste, health, colour and aroma of Sri Lankan mango fruits increase during ripening
- Concentration of biochemical compounds of Sri Lankan mango fruits is higher than commercial mango cultivars
- Lower ripening temperature extend the shelf-life whilst maintaining the nutritional and quality parameters, which is instrumental for export market
- Exclusive aroma compounds of Sri Lankan mango fruits attract consumers and seed dispersers, and are used to differenciate the cultivars

1.2.3 Aim

To improve the understanding of spatial distribution and temporal changes in biochemical constituents in pre- and postharvest Sri Lankan mango cultivars during maturation and ripening in order to identify optimum harvest maturity and storage potential.

1.2.4 Objectives

- To determine the biochemical constituents in different spatial sections of the pre-climacteric Sri Lankan mango fruits at different maturity stages in order to identify the optimum harvest maturity
- To determine the temporal changes of biochemical compounds and quality parameters in post climacteric Sri Lankan mango fruits during ripening at low and high temperatures to identify the optimum ripening stage
- To develop a new method to quantify volatile compounds from postharvest Sri Lankan mango fruits during ripening at low and high temperature
- To compare the biochemical profiles of postharvest Sri Lankan mango cultivars with commercial cultivars in order to potentially differentiate Sri Lankan mango cultivars

1.3 Structure of the thesis

The thesis is arranged into eight chapters. Chapter one is the introduction, and includes the background of the study, aim and objectives and structure of the thesis. Background describes the usefulness of mango fruits and justifies the importance and need of this study. Chapter two is a review of existing literature, which describes the past studies relevant to this thesis. Past studies demonstrate that although mango fruits have been extensively studied globally, there is a real paucity of published information on mango cultivars endemic to Sri Lanka. Chapter three details the research carried out on pre-climacteric Sri Lankan mango cvs. Willard, Karutha Colomban, Malgova, Vellai Colomban and Ampalavi in order to understand the spatial variation and temporal

changes of biochemical compounds at different maturity stages. The results from this work have been published as follows:

- Thanaraj, T., Terry, L. A. and Bessant, C. (2009). Chemometric profiling of pre-climacteric Sri Lankan mango fruit (*Mangifera indica* L.). *Food Chemistry* 112, 851-857 (Appendix B.2)
- Thanaraj, T. and Terry, L. A. (2008). Spatial and temporal profile of non-structural carbohydrates in pre-climacteric Sri Lankan mango (*Mangifera indica* L.) fruit. November 4 – 7, 2008, Berlin, Germany. Oral presentation (Appendix B.3). *This paper has now been published in Acta Horticulturae 858, 137-142.*

Chapter four describes the research conducted on postharvest Sri Lankan mango cultivars (Willard, Karutha Colomban and Malgova). Mango fruits were harvested and air freighted (2007) from Sri Lanka to Cranfield University and ripened at 32^{o}C for 4 days. Peel and pulp samples were analysed to understand the temporal changes in biochemical constituents during ripening. The results from this work have been published as follows:

- Thanaraj, T. and Terry, L. A. (2009). Temporal change in biochemical compounds during postharvest ripening of Sri Lankan mango fruit (*Mangifera indica* L.). April 8 – 12, 2009, Antalya, Turkey. Oral presentation (Appendix B.4). *This paper has now been published in Acta Horticulturae 877, 1183-1190*
- Thanaraj, T., Terry, L. A. and Thivyatharsan, R. (2008). Effect of ripening on chemometric profile of taste-related compounds in mango cv. Willard fruit. *Proceedings of the Annual Research Conference*, 7th Annual Research Conference of Eastern University, Sri Lanka during November 20 – 23, 2008

Chapter five describes the work conducted on postharvest Sri Lankan mango cultivars (Willard and Karutha Colomban). Mango fruits were air freighted (2008) from Sri Lanka to Cranfield University and ripened at 20^{o}C and 30^{o}C for 6 days with a sudden change of temperature from 20^{o}C to 30^{o}C and 30^{o}C to 20^{o}C at day 3. Peel and pulp samples were analysed to understand the temporal changes of biochemical constituents during ripening at different temperature (low and high) with a temperature shock at day 3. An abstract from this work was accepted for an oral presentation at the Fav Health 2009, France (Appendix B.9) and the rest of the results have been prepared for subsequent publications (Appendix B.7).

Chapter six describes a new method developed to quantify the volatile profile of Sri Lankan mango cultivars (Willard, Karutha Colomban and Malgova) ripened at different temperatures. Volatile compounds from the peel and pulp samples were extracted using HS-SPME and quantified using gas chromatography coupled with flame ionization detector (GC-FID) using external standards. An abstract from this work was accepted for an oral presentation at the international mango symposium, China (Appendix B.8).

Chapter seven includes the general discussion, conclusion, suggestions and recommendations for future work, and a comparative study of Sri Lankan mango cultivars with other cultivars respective to their biochemical composition (TSS, sugars, starch, non-volatile organic acids, ascorbic acid, titratable acidity, total phenolics, flavonoids and volatiles).

Chapter eight is the literature cited followed by Appendices (Appendix A, B, C and D).

CHAPTER TWO

Literature Review

2.1 Mango

2.1.1 History, distribution, production and cultivars

Mango (*Mangifera indica* L.) is one of oldest fruits cultivated by man. Mango belongs to the genus *Mangifera*, consisting of several species of tropical fruits in the family Anacardiaceae. Mango fruit is believed to be a native of the Indo-Burma region and was introduced to Malaysia and East Asia by Indian traders. The fruit was grown in the East Indies before the earliest visit of the Portuguese and was introduced by the Portuguese to West Africa and Brazil in the early 16th Century, and then mango cultivars were spread to the West Indies, Jamaica and Mexico (Morton, 1987). Polyembryonic and monoembryonic mangoes are geographical races distributed throughout Southeast Asia and India-Burma region, respectively. According to FAO statistics for the year 2007, at least 87 countries produce mangoes, continuing a smooth increase totalling slightly over 28.51 million tonnes per annum. Asia is the main producer of mango with 76.9% of the total world production followed by the Americas (13.8%) and Africa (9%) (FAO, 2007). However, world mango production is forecast to rise up to 30.7 million tonnes by 2010, which will account for more than 50% of global tropical fruit production (FAO, 2005).

In general, mango production in Sri Lanka is seasonal. The majority of mango fruits are grown in the Wet and Intermediate Zones and bloom in January-March and are harvested in May–July for the Yala crop. Mangoes in the Dry Zone bloom between July-September for the main Maha crop and are harvested in November-January (Figure

2.1). Mangoes are commonly grown as a rain-fed crop, therefore the flowering and harvesting time may shift considerably depending on rainfall distribution patterns. Plentiful production of in-season mangoes leads to low prices whilst production drops between seasons resulting in higher prices. Therefore, off-season mango production techniques need to be improved (Table 2.1A and B) (Peiris and Senevirathna, 2001).

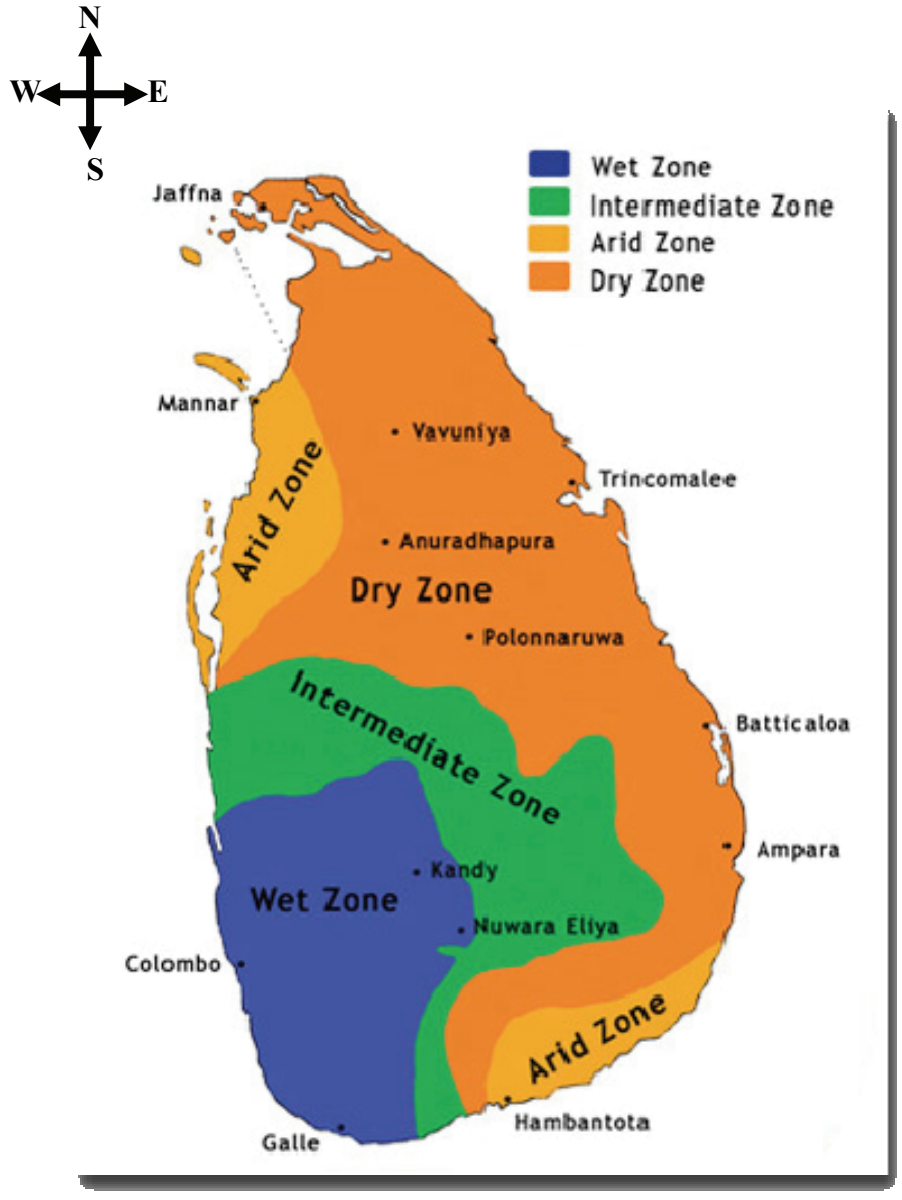

Figure 2.1. Agro-ecological regions of Sri Lanka (Source: Department of Meteorology, Sri Lanka)

Table 2.1. Weather data of Batticaloa, Sri Lanka for the year 2007 (A) and 2008 (B)

(Dry Zone, East of Sri Lanka: Figure 2.1)

A

Month	Mean temperature (^{o}C)		Mean total rainfall (mm)	Mean relative humidity (%)
	Daily minimum	Daily maximum		
January	23.2	27.8	210.3	85
February	23.6	28.6	128.4	81
March	24.3	30.0	89.0	80
April	25.3	31.6	55.0	75
May	25.7	33.0	39.3	74
June	25.6	34.0	23.9	70
July	25.3	33.4	41.4	69
August	25.1	33.1	48.5	72
September	24.8	32.2	67.0	73
October	24.3	30.9	180.0	78
November	23.8	29.3	349.6	83
December	23.5	28.1	418.5	86

Source: Department of Meteorology, Sri Lanka

B

Month	Mean temperature (^{o}C)		Mean total rainfall (mm)	Mean relative humidity (%)
	Daily minimum	Daily maximum		
January	23.6	27.6	288.6	86
February	22.9	28.8	140.4	82
March	24.0	39.6	94.5	80
April	25.8	30.8	51.7	77
May	25.9	31.9	42.0	73
June	26.0	33.0	32.9	71
July	25.8	33.6	39.1	69
August	25.2	33.3	42.8	73
September	25.0	32.2	72.6	74
October	24.2	31.5	120.8	80
November	24.0	29.8	274.3	85
December	23.6	28.0	305.6	86

Source: Department of Meteorology, Sri Lanka

Total mango production in Sri Lanka is around 96,500 tonnes with a world ranking of 27 out of 87. Mango is cultivated on about 26,000 ha of land from three major agro-ecological regions (Figure 2.1). However, the overall lower production volume, lack of proper storage facilities, transport facilities and relatively higher postharvest losses limit the processing and export possibilities (FAO, 2005). Though mango production is distributed throughout Sri Lanka, dry zone leads the production as it occupies the major part of Sri Lanka. Dry zone mango fruits have unique colour, flavour, taste and aroma properties, which attain greater consumer demand in local markets.

There are hundreds of mango cultivars distributed around the world (Nakasone and Paull, 1998). However, Western Malaysia has the highest species diversity (28 species). Mangoes are mainly clustered into unimproved, improved tropical and improved sub-tropical cultivars. Peach and Sabre (polyembryonic) are unimproved cultivars, and are usually turpentine flavoured and contain higher fibre, poor external colour and a higher susceptibility to disease than commercial cultivars. On the other hand, the improved tropical cultivars produce turpentine flavour-free and fibreless fruits (Snyman and Schroeder, 1992). However, such improved cultivars result in poor yield and unacceptable external colour. The improved sub-tropical cultivars (Keitt, Sensation and Tommy Atkins cultivated mainly in Brazil and Florida) generally have desirable colour and good eating quality (Nakasone and Paull, 1998). However, these cultivars are often criticized by many for being bland and have been bred for shelf-life and postharvest durability at the expense of flavour and taste attributes.

A number of mango cultivars have been cultivated in Sri Lanka. Since the country has vast climatic variation, cultivars differ among agro-ecological regions.

Cultivars such as Willard, Karutha Colomban, Vellai Colomban, Chempattan, Malgova and Ampalavi are commonly grown in the low country (Dry zone) that experiences more or less a tropical climate whilst cvs. Karutha Colomban, Vellai Colomban, Willard and Betti Amba are grown in the mid country (Intermediate zone). Cultivars such as Vellai Colomban, Peterpassand, Dambara and Gira Amba are grown in the up country (Wet zone) which has a temperate climate (Anon, 2009a) (Figure 2.2). However, mango cvs. Willard, Karutha Colomban, Malgova, Ampalavi and Vellai Colomban are very popular in Sri Lanka since they are widely appreciated for their aroma and taste. This said, cultivars are grown throughout the country and occupy the domestic market regardless of seasonal differences.

A **B** **C** **D** **E**

Figure 2.2. Prominent Sri Lankan mango cultivars: Willard (A), Karutha Colomban (B), Malgova (C), Ampalavi (D) and Vellai Colomban (E)

2.1.2 Morphological description of mango

The height of mango trees ranges between 10 to 40 m with an evergreen, symmetrical and rounded canopy. Alternately arranged leaves are lanceolate in shape and leathery in texture. The inflorescence (6 to 41 cm) is a long and many-branched

panicle, borne on shoot terminals (550 to 4000 flowers). The shape (nearly round, oval, ovoid-oblong), size (2.5 to 30 cm length) and colour of mango fruit are mainly dependent on genotype. Ripe mango fruit may vary in colours (greenish, greenish-yellow, yellow, red, orange or purple) and weigh up to 2.3 kg. The pale yellow to orange coloured edible portion (pulp) of the mango fruit is surrounded by the leathery skin. The fruit generally has a single, trodden and kidney-shaped seed (Figure 2.3). Even though fruits ripen on the tree, they are usually picked at mature green stage (defined stage) for the market (Medina and García, 2002). A fruit harvested at mature (having completed natural growth and development) stage ensures that the fruit quality will meet or exceed the consumer acceptance at the time of consumption. Mango fruit harvested at immature stage will not be able to achieve the final quality acceptable by consumers. Immature, partially-mature and fully mature green are three distinguishable maturity stages identified during mango fruit development (Slaughter, 2009). Mango fruits are harvested at fully mature green stage (still firm) in order to facilitate successful marketing with conventional packaging and postharvest handling practices since mango takes about 8 to 10 days at 25^{o}C (Lakshminarayana, 1980) to ripe. However, newly developed suspended tray packages (Thompson *et al*., 2008) may allow harvesting mango fruits at more mature or partially-ripe stage if transit time is not too long (Slaughter, 2009). An allergenic urushiol, 5-heptadecenylreorcinol is identified as the main irritating constituent of mango sap (acrid juice) present in the stalk and unripe fruits (Medina and García, 2002). Prusky and Goldman (2009) reported that a mixture of 5-(12-*cis*-heptadecenyl)) and 5-pentadecylresorcinol was isolated from the peel of unripe mango (*Mangifera indica* L.) fruits, which is an antifungal agent related to the latency of *Alternaria alternate*. The concentration of the 5-substituted resorcinols

in peels of unripe fruits was about 200 µg g^{-1} FW and it decreases during ripening to about 100 µg g^{-1} FW. This decrease may enhance the development of previously latent *Alternaria alternate* infections in ripe fruits. In addition, it is believed that contact of sap with the mango skin during harvesting may lead fungal infections like stem end rod during postharvest ripening.

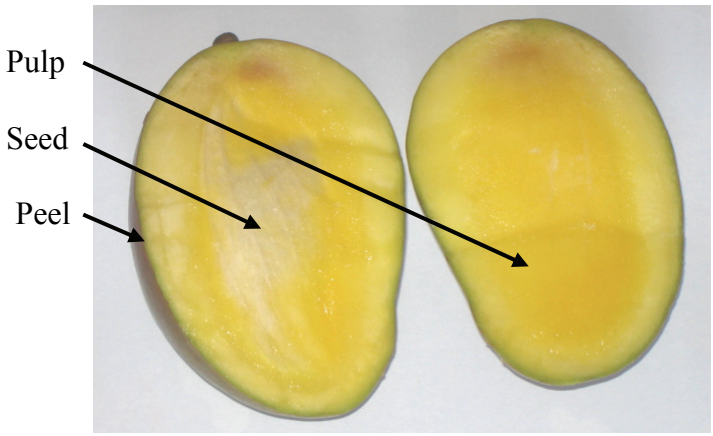

Figure 2.3. Fully mature mango cv. Willard fruit

2.1.3 Fruit development and harvest maturity

The final quality of mango depends not only on the physiological processes during ripening, but also on fruit development and maturation. Identifying reliable indices of harvest maturity is being sought as this affects subsequent postharvest ripening, fruit quality (Peacock *et al.*, 1986; Medlicott *et al.,* 1988), response to various postharvest operations (Esguerra and Lizada, 1990), processing quality (Kapur *et al.,* 1985) and ultimately market acceptability.

Mango fruit follows a simple sigmoid pattern of growth (Kasantikul, 1983; Tandon and Kalra, 1983). The rapid growth is characterised by the increase in alcohol-

insoluble solids (especially starch) (Tandon and Kalra, 1984). The accumulation of starch is increased due to an enhanced amylase activity in mango cvs. Dashehari (Tandon and Kalra, 1983) and Haden (Fuchs *et al.,* 1980). The increase in dry matter is used as an index of maturity in mango cv. Kensington Pride fruit (Baker, 1984) and also considered as the basis for the use of specific gravity as a maturity index in mango cvs. Alphonso (Subramaniyan *et al.,* 1976), Dashehari (Kapur *et al.*, 1985) and Carabao (Cua and Lizada, 1990).

The maturity of mango fruit is commonly judged by broadening of the shoulder and the colour change from green to yellow. The colour of inner flesh (closest to the seed) changes to yellow prior to the external colour breaking (Medina and García, 2002). The period from fruit set to fully mature stage ranges from 10 to 28 weeks; however it depends on cultivar and climate. Besides the morphology, other factors such as mass, sugar, acid and starch content are also used as determinants to optimise the harvest maturity. Pulp colour (cream colour turns to light yellow on maturity) and the hardness of the seed stone are considered as important indications to observe maturity in mango (Anon, 2009b).

All mangoes should be harvested at harvest maturity (physiologically mature stage). The harvest maturity is determined according to storage and shipment time required but harvesting criteria varies with consumption patterns of local people and distance of transportation (Lizada, 1993). An appropriate harvest maturity is attained twelve weeks after fruit set in mango cvs. Dashehari and Langra whilst fifteen weeks are required for cvs. Chausa and Mallika in the same environment. In general, four maturity stages have been identified in mango fruit such as fruit shoulders in line with stem-end (green colour), shoulders outgrow from the stem-end (green colour), shoulders

outgrow from the stem-end (light colour) and flesh becomes soft and develops blush. Fruits harvested at any of the above stages ripen well but those harvested at later stages result in better taste and flavour (Gunjate, 2006). However, fruits harvested ripe on the tree are generally better in terms of taste, aroma and nutritional quality but have very poor shelf-life and thus are impractical for large scale export and distribution. A balance is clearly needed between shelf-life and attaining desired taste profiles.

2.1.4 Mango ripening and the role of ethylene in ripening and fruit development

Fully mature mango fruits show a significant decrease in starch and a distinct yellowing of the pulp during the later stage of maturation (Wang and Shiesh, 1990). Such changes are accompanied by a decline in pulp rupture force in several cultivars (Cua and Lizada, 1990; Seymour *et al.,* 1990). These observations indicate that some changes are associated with ripening induced prior to harvest maturity and point to the possibility of ethylene (C_2H_4) production prior to detachment.

Although ethylene production of mango fruits is comparatively lower (< 5 $\mu l\ g^{-1}\ h^{-1}$; Ketsa *et al*., 1999) than other fruits, it influences significantly on the ripening of mango fruits. Ethylene is also believed to be associated with abscisic acid and fruit drop in pre-climacteric mangoes (Leon *et al.,* 2002). An increase in pre-climacteric ethylene production increases the level of abscisic acid in the neck whilst a drop in ethylene production reduces the abscisic acid levels. Accumulation of abscisic acid induces pre-climacteric fruit drop. A sudden increase of ethylene production in ripening mangoes has normally been modified by refrigeration and heat pre-treatment. The ethylene peak in refrigerated fruit appears before the climacteric period whilst it is delayed or inhibited in pre-heated fruit. Mango fruits are treated using hot water bath or steam at 52^{o}C -

55^{o}C for 5 minutes prior to ripening either at ambient or modified ripening conditions. Decrease of ethylene production may be due to the inhibition in 1-aminocyclopropane-1-carboxílic acid (ACC) synthesis and 1-aminocyclopropane-1-carboxílic acid oxidase (ACCO) activity. The ethylene levels can be considered as a biochemical index of chilling injury in mangoes (Leon *et al.,* 2002). Burg and Burg (1962) reported that mature green mango fruit produce considerable ethylene (1.87 µl l^{-1}) even while attached to the tree. Ethylene is not detected in fully mature mango cv. Carabao, however ethylene production is expected to resume when the fruit approaches colour break (Cua and Lizada, 1990).

2.1.4.1 Ethylene

Ethylene is one of the endogenous growth regulators and it is naturally produced from plant tissues. However, the role of ethylene was confirmed after using it for several years in plant growth and development manipulations. The biological activity of ethylene led it to be considered as a plant hormone produced up to 500 nL g^{-1} h^{-1}, but ethylene is active as low as 10 to 100 nL g^{-1} h^{-1} (Peech *et al*., 1992). Several studies have revealed the intervention of ethylene in fruit ripening, seed germination, leaf and flower senescence and abscission, root growth and development, and somatic embryogenesis (Abeles *et al*., 1992). However, ethylene is also reported to be synthesised in response to stress caused by wounding, very low and very high temperature, flooding, drought, treatment with other hormones, heavy metals and attack by pathogens (Peech *et al*., 1992).

Ethylene production has been defined in two systems in plants, whereas system one functions during normal growth and development, and stress responses. This is an

auto-inhibitory process that said exogenous ethylene inhibits synthesis, and inhibitors of ethylene action can stimulate ethylene production. The system two operates during floral senescence and fruit ripening, in contrast it is auto-catalytic process as stimulated by ethylene, and inhibitors of ethylene action inhibit ethylene production (Figure 2.4) (Barry and Giovannoni, 2007).

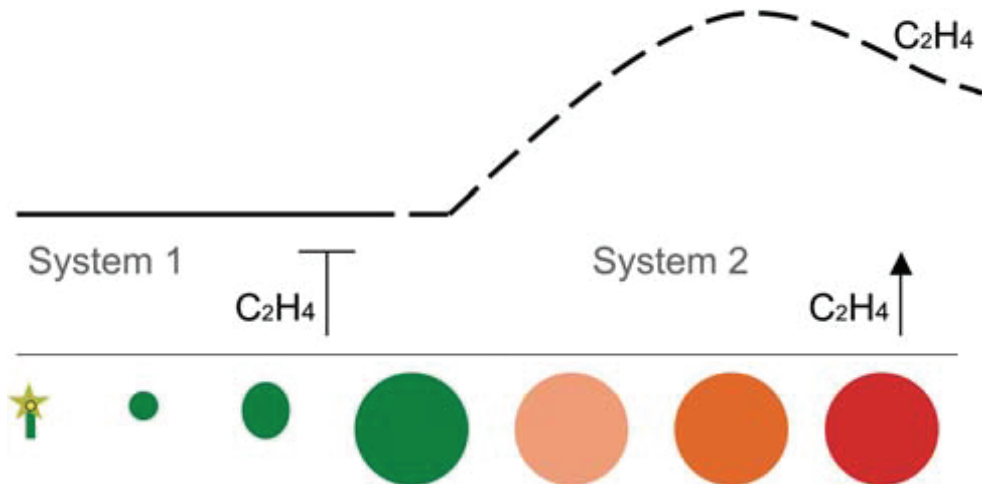

Figure 2.4. System 1 (auto-inhibition) and system 2 (auto-catalytic) ethylene synthesis during fruit development and ripening in tomato (Barry and Giovannoni, 2007)

2.1.4.2 Ethylene biosynthesis

Ethylene biosynthesis pathway begins from methionine in 3 main steps, whereas step 1 is the conversion of methionine to S-adenosyl-L-methionine (SAM), which is catalyzed by the enzyme SAM synthetase. Step 2 is the formation of 1-aminocyclopropane-1-carboxylic acid (ACC) from SAM via ACC synthase (ACS) activity and step 3 is the conversion of ACC to ethylene, which is catalyzed by ACC oxidase (ACO). The formation of ACC may also leads to produce 5¢-

methylthioadenosine (MTA) which is recycled to produce new methionine molecule through methionine pathway (Figure 2.5). Increase in respiration during ethylene synthesis provide ATP required for methionine cycle that increase the rates of ethylene production without high levels of intracellular methionine (Zegzouti *et al*.,1999; Alba *et al*., 2005; Lin *et al*., 2009).

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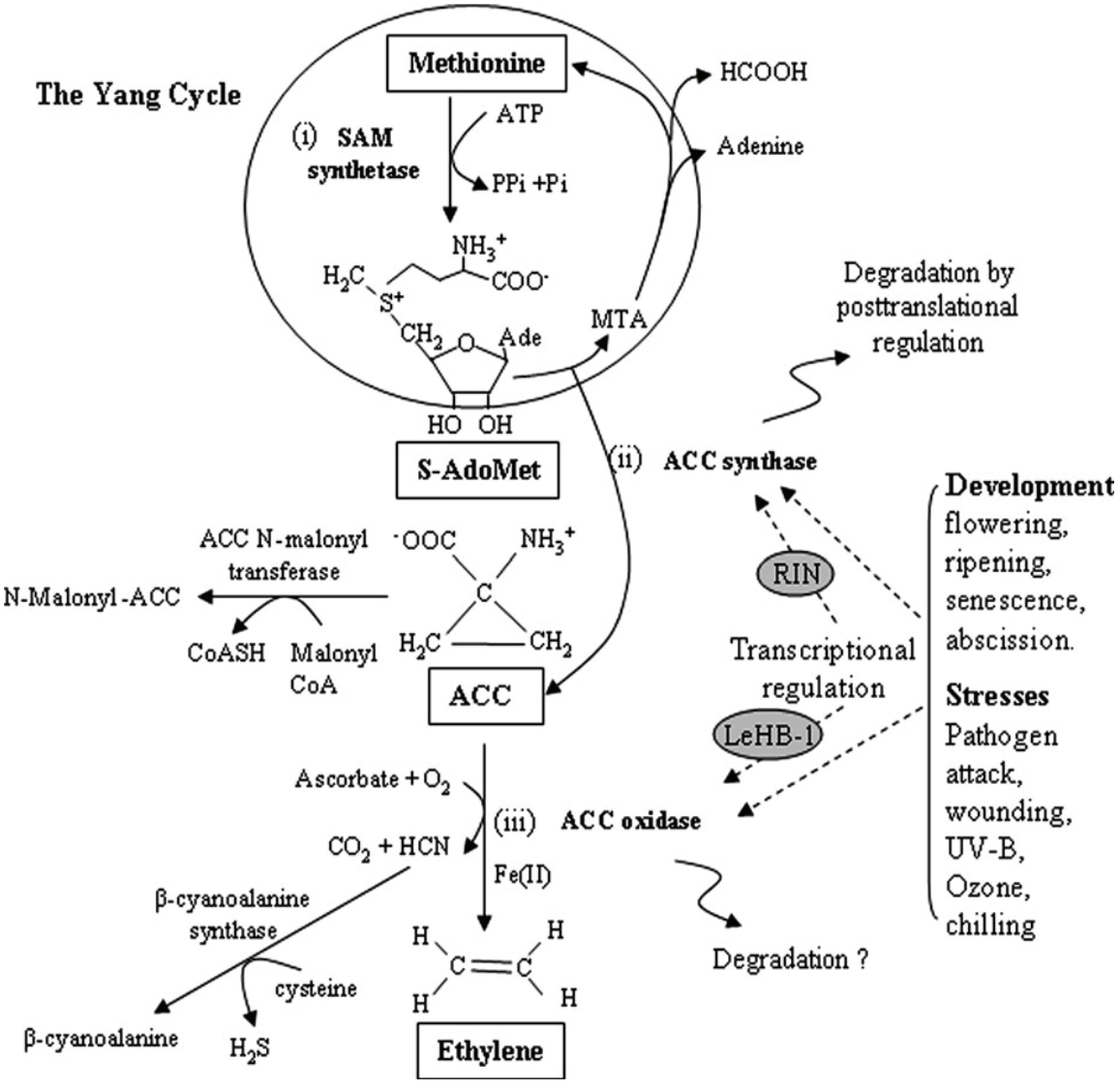


Figure 2.5. Ethylene biosynthesis pathway and its relationship to the methionine cycle. ACC: 1-aminocyclopropane-1-carboxylic acid; ACCS: ACC synthase; ACCO: ACC

oxidase; Ade: adenosine; ADP: adenosine 5'-diphosphate; ATP: adenosine 5'-triphosphate; CMB: 2-keto-4-metilbutyrate; MTA: 5'-metilthioadenosine; MTR: 5-metilthioribose; MTR-1-P: 5-metilthioribose-1-phosphate; N-MACC: N-malonyl ACC; NMT: N-malonyl transferase; SAM: S-adenosyl methionine; S-AdoMet: S-adenosyl methionine (Lin *et al*., 2009)

2.1.4.3 Climacteric and non-climacteric fruits

Climacteric fruits (mango, tomato, apple, peach, banana, etc.) have response to enhanced ethylene production and associated increase in respiration rate at the onset of ripening, whereas fruits that do not produce elevated ethylene are called as non-climacteric fruits (citrus, grape, strawberry, etc.). Melon and capsicum species can be both climacteric and non-climacteric but non-climacteric fruits show enhanced ripening response to exogenous ethylene (Barry and Giovannoni, 2007).

Ethylene is required for many fruits to enhance ripening process. Absence or reduced levels of ethylene leads incomplete ripening process and subsequently causes the product unpalatable. Ripening is a one-way process, therefore once initiated the beneficial effects of ethylene can soon be overshadowed by stimulating over ripening and decay. In that sense, considerable effort is made to control ethylene effects in fruits, vegetables and ornamental crops during their postharvest storage (Barry and Giovannoni, 2007).

The ethylene production accelerates once the apple begins to ripe, ethylene concentration is very low (< 0.15 ppm) in apple before the initiation of ripening but it increases rapidly well over 100 ppm during the ripening process (100-fold in 2 days). This dramatic increase of ethylene production is called the climacteric and often

associated with a increase in respiration (Figure 2.6). This burst in ethylene production provides a definite dividing line between pre-climacteric and post climacteric stages of apple and pears. Generally, detached fruit begins the climacteric before the fruit is still attached to the tree, therefore if fully mature fruit (closer to begin climacteric) is picked that may shorten the time of ethylene production **(**Kupferman, 1986**).**

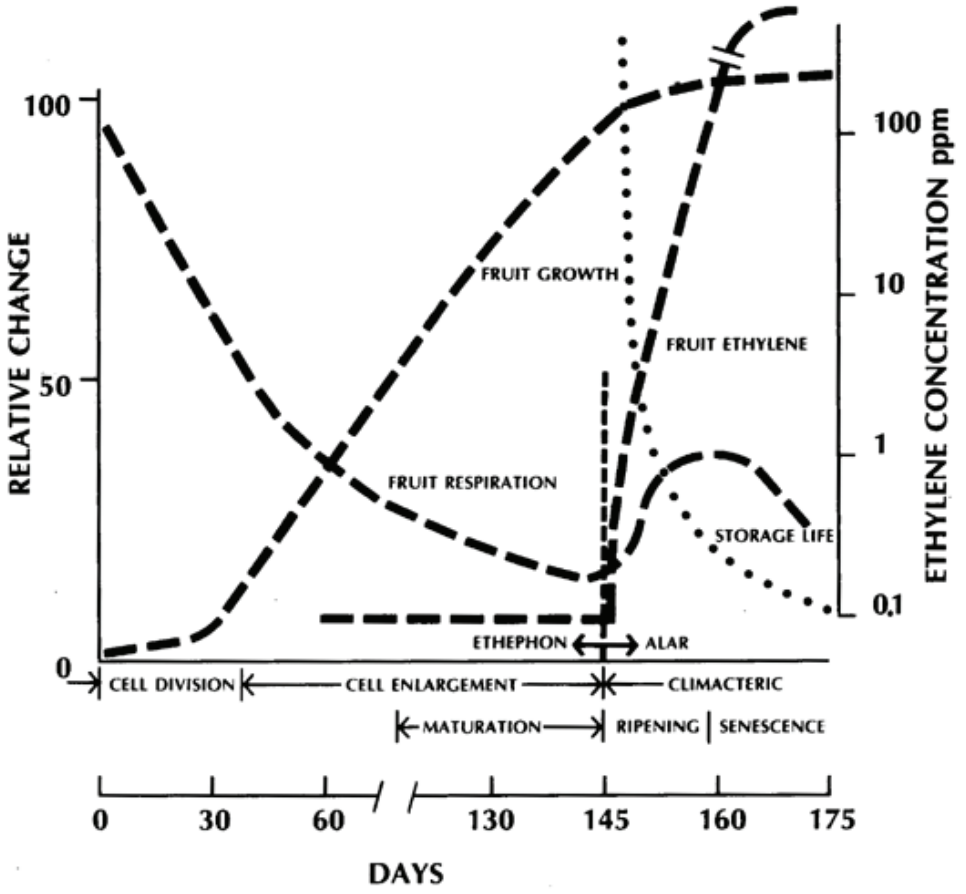


Figure 2.6. Growth and development of apple and pear fruits in relation to the effects of ethylene, ethephon, alar, and ripening (Dilley, 1981)

2.1.4.4 Mango fruit development stages

According to figure 2.6, ethylene production is very low and consistent until the full mature stage of mango fruit (120-140 DAFB). Ethylene production abruptly

increases after the harvest and reaches the maximum at the end of ripening period (15 days from the harvest). However, the respiration rate reduces during fruit maturation but increases after the harvest with a climacteric peak at the end of ripening period. Both ethylene production and respiration rate reduce with the onset of senescence (Figure 2.7).

Immature 70-90 DAFB | Half Mature 90-120 DAFB | Fully Mature 120-140 DAFB | Fully Ripe 150-155 DAFB | Over Ripe 155 DAFB

Figure 2.7. Different stages of mango cv. Willard fruit development

2.1.4.5 Ethylene receptors

The transduction pathway from ethylene perception to gene activation has been studied much in recent years. Ethylene is bound by a membrane-localised receptor and its action very often involves transcriptional activation. The N-terminal domain of the receptor protein is responsible for binding of ethylene and a protein kinase is the intracellular portion of the protein, which is activated upon binding of ethylene. The protein kinases are termed two-component system as they composed of a sensor and a response regulator. The first two-component receptor discovered in plants was the ethylene receptor ETR1 (ethylene receptor 1) from *Arabidopsis thaliana* L. and then in tomato (Bleecker and Kende, 2000).

Six receptors such as LeETR1, LeETR2, NR (LeETR3), and LeETR4, 5, and 6 (Klee, 2004) have been identified so far in tomato whilst Arabidopsis has five receptors (ETR1, ERS1, ETR2, ERS2, and EIN4; Schaller and Kieber, 2002) (Lin *et al*., 2009). Ethylene receptors found in Arabidopsis are considered to be functionally redundant, however tomato ethylene receptors such as NR (LeETR3), LeETR4 and LeETR6 are preferentially expressed in fruits and are believed to have unique roles during ripening (Tieman *et al*.**,** 2000; Kevany *et al*.**,** 2007; Lin *et al*., 2009).

2.1.4.6 Ethylene inhibitors

Several chemical compounds have been developed to stop or reduce ethylene action in fruits, vegetables and ornamental crops. These compounds are believed to act through binding the ethylene receptors (Sisler, 2006). The 1-Methylcyclopropene (1-MCP) is an effective inhibitor of ethylene responses used in the trade names of EthylBloc and SmartFresh for ornamental and edible horticultural products, respectively (Watkins, 2006). The 1-MCP (C_4H_6) has physical similarity to ethylene allowing it to bind the ethylene receptors and inhibiting the normal action of ethylene, thus extending the storage life of fruits. However, 1-MCP has been widely used as a supplement to proper postharvest temperature management or controlled atmosphere storage (Sozzi and Beaudry, 2007). Watkins (2008) reported that the application of 1-MCP is most suitable for fruits like apple than fruits (mango, papaya, etc.) that have a change in texture between harvest and consumption as normal softening associated with ripening. The crunchy texture of apple is preferred to be maintained from harvest to consumption in order to attain better consumer acceptance.

2.1.5 Harvesting and handling of mangoes

Mango fruit should be harvested in the morning and kept under shade. Care must be taken to avoid the fruits falling on the ground directly in order to reduce injuries. Mangoes picked with (8 to 10 mm) long stalks are usually free from sap burn on the skin. The said fruits are generally less prone to stem-end rot and other postharvest diseases. Fruits harvested by using sticks are commonly injured or bruised due to impact, subsequently those fruits result in decay, poor quality and command a lower price. Traditional harvesting methods are time consuming and result in more losses at harvest in addition to greater postharvest losses. To overcome these problems, many types of portable mango harvesting devices with picking pole and cutting mechanism have been developed in mango producing countries (Thanaraj and Risvy, 2005; Rathore, 2007).

2.1.6 Grading and packaging

Bigger mango fruit usually takes 2 to 4 days more to ripe than smaller ones. Therefore, mixing of larger and smaller fruits is avoided to optimise uniform ripening. Grading of fruits based on their size, colour, weight and maturity can be beneficial to both producer and consumers. Immature, over ripened, damaged and diseased fruits are discarded. Mango fruits have traditionally been packed in wooden boxes for local market transport. However, fruit quality is affected (shrinkage, loss in weight and colour) due to excess ventilation. To overcome these problems, carton fibre board (CFB) boxes (5 kg and 10 kg capacity) have been developed and used extensively (Rathore, 2007). Cushioning material such as paper scraps and newspapers are generally

used for packaging of fruits since these reduce bruising and spoilage. Polyethylene (LDPE) lining is also beneficial since it reduces shrinkage of fruits during storage by maintaining the humidity. Individual packaging (unipack) of fruits using newspaper or tissue paper in a honeycomb structure may reduce spoilage and improve ripening (Anon, 2009b). For export purposes, the fruit stem (pedicel) is cut approximately to 1 cm from the fruit. Then the fruits are kept upside down in specially knitted pallets for about two hours to facilitate the flow of latex out from the fruit. For export purposes, mangoes are generally classified into three grades i.e. Category-I (200 to 250 g), Category-II (251 to 300 g) and Category-III (300 to 350 g) according to the fruit weight (Rathore, 2007; Anon, 2009b).

2.1.7 Storage of mango

Storage extends the shelf-life of fruits for consumption, marketing and transportation for long distances. Mature-green mango fruits can be kept at room temperature for about 4 to 10 days, but that depends upon the cultivar and ambient temperature. Shelf-life of mango fruits can generally be extended by pre-cooling, chemical treatments and low temperature. Mango fruits are generally pre-cooled to 10^{o}C to 12^{o}C (temperature < 10^{o}C may cause chilling injury) before storing at an appropriate temperature. Mango cvs. Dashehari, Mallika and Amrapali are stored at 12^{o}C, but cvs. Langra and Chaunsa are stored at 14^{o}C and 8^{o}C, respectively at 85 to 90% relative humidity for the commercial market (Singh *et al*., 2004). However, the fruits can be stored for 3 to 4 weeks at a lower temperature. Chilling injury can be overcome by storing fruits in ventilated polythene bags. Calcium infiltration (dipping in 4-5% calcium chloride) may extend the storage life of mango fruits. Lower calcium level of

mango fruit is associated with reduced postharvest life and physiological disorders (Van Eeden, 1992; Wills *et al*., 1998). Calcium treatment reduces the respiration and ethylene production and thus eventually delays the onset of mango ripening (Van Eeden, 1992). Since pre-harvest spray and postharvest treatment of calcium are considered as practically difficult and can have limited success, calcium chloride dips (4 or 8% of $CaCl_2$) is commonly practised as it has reported to increase the ripening time of mature mangoes (Mootoo, 1991). However, mango fruits in Sri Lanka are kept under shade in ambient condition for further ripening. Since Sri Lankan mango fruits are primarily targeted for the domestic market, there is no need to adopt any storage structure by modifying the normal atmospheric conditions as this may involve in relatively higher operational cost. However, if Sri Lankan growers want to increase exports then many of the practices outlined above may need to be adopted.

Controlled atmospheric (CA) and modified atmospheric (MA) storages of tropical fruits (including mango) reduce respiration rate, lower ethylene biosynthesis, delay ripening and maintain the quality of fruits during long term storage, therefore both CA and MA storages may extend the shelf-life of mango fruits. The CA means that a defined mixture of gases is maintained or controlled over time by external apparatus or internal chemical reactions whilst in the MA, gas atmosphere is modified by direct injection of gases (CO_2 or N_2) or evacuating air from the package or interaction between the package/storage structure contents and the inside air causing the storage atmosphere changing over time. However, the period of extension of shelf-life varies with temperature, atmosphere and genotype. Chervin *et al.* (2000) reported that although CA storage is widely used to extend the shelf-life of mangoes, consumers have concern about the poor aroma (unpleasant and different from natural fruit aroma) of CA-stored

fruits. CA and MA are not commonly used to store fruits in tropics, but are used extensively for their transport (local and international) (Kader, 1983; Yahia and Singh, 2009). Extending the shelf-life of Sri Lankan mango fruit for export using controlled atmospheric storage could be recommended for long term storage. However, no research work has yet been reported on the effects of CO_2 and O_2 levels during controlled atmospheric storage on ripening and quality of Sri Lankan mango fruits. Increased CO_2 concentrations and/or decreased O_2 concentrations are believed to alter the aroma and flavour of various fruits in the storage atmosphere (Kader, 1993; Yahia, 1998). Mango cv. Alfonso stored above 15 kPa CO_2 can cause fermented odour (Lakshminarayana and Subramaniyam, 1970) whilst CO_2 concentrations above 10 kPa induced high levels of ethanol production in the pulp of mango cv. Kensington Pride (O'Hare and Prasad, 1993). However, CA storage has been successfully applied to various mango cultivars including Alfonso, Raspuri (Kapur *et al.,* 1962), Keitt (Hatton and Reeder, 1965), Irwin (Maekawa, 1990), Rad (Noomhorm and Tiasuwan, 1995), Kent, Tommy Atkins (Lizana and Ochagavia, 1997) and Kensington Pride (Lalel *et al.,* 2001), but the optimum concentration of CO_2 and O_2 may vary from cultivar to cultivar (Kader, 1983; Yahia, 1998).

Mango cvs. Irwin and Tommy Atkins fruits can be stored at 10^oC for 3 weeks, but cvs. Haden and Keitt may be affected by chilling injuries at this temperature (Hardenburg *et al*., 1986). Mango cvs. Alphonso and Raspuri fruits can effectively be stored for 35 and 49 days, respectively, at 7.5 kPa CO_2 at 7.2 to 10^oC whilst mango cv. Haden fruits can successfully be stored for 30 days at 6 kPa O_2, 10 kPa CO_2 and 8^oC (Bleinroth *et al*., 1977). Therefore, requirements of temperature and the composition of O_2 and CO_2 for extending the shelf-life differ greatly from cultivar to cultivar.

2.1.8 Postharvest physiology, disorders, pests and diseases

Postharvest deterioration of a fruit can be caused by several factors such as physiological change, physical damage, chemical injury and pathological decay. Mechanical damage such as bruising and puncturing generally causes localised softening and secondary microbial infection in mango fruit during harvesting and postharvest handling. Chilling injury may result in poor flavour development, uneven ripening and discoloration of mango fruit (skin and pulp). Physiological disorders such as jelly seed, soft nose and stem-end cavity can also cause considerable losses in the mango industry (Raymond *et al*., 1998). Postharvest chemical treatments (fungicides, $CaNO_3$, etherel, bavistine, etc.) are not recommended as these may cause reactions on fruit itself or leave undesirable residues on fruit surfaces.

Higher degrees of perishability was observed for ripe mango fruits and this is the major constraint in the local and export market. Therefore, diseases such as anthracnose (*Colletotrichum gloeosporioides*), black spot (*Alternaria alternata*) and stem-end rot (*Dothiorella* or *Botryodiplodia*) may develop in mango fruits within 6 to 10 days after harvest in a tropical climate. Such diseases are considered as economically important in the global mango industry. Postharvest disease development in mango fruit cannot be arrested completely even with cold temperature storage, but can be suppressed to some extent using various postharvest technologies (Prusky *et al.,* 2004; Prusky *et al.,* 2006).

2.1.9 Marketing and international trade

The global mango trade has increased by about three-fold during the past decade. The current mango trade has been estimated at around 650,000 tonnes per

annum. Though mango fruits have mainly been exported from the Mexico, Brazil, Pakistan, India, South Africa, Australia, Kenya, Puerto Rico, Jamaica, Thailand and Peru, still there is high demand for good quality mango fruits. Mexico (largest mango exporting country) supplies around 41% of the world market demand, followed by the Philippines (7.8%) and Pakistan (7.6%) (Table 2.2). The European Union, United States and Japan are the prominent importers of fresh mango fruits (Rathore, 2007; Anon, 2009b). Mango fruits are imported by most developed countries at a regular supply throughout the year but are still based to a degree on the mango seasons of producing countries (Anon, 2005).

Table 2.2. Global export of mango during the past five years (1999/00 to 2003/04)

Year	Export volume (000 tonnes)	Export value (000 US$)
1999/00	47.60	11,576
2000/01	53.44	17,005
2001/02	47.54	14,036
2002/03	58.84	17,626
2003/04	77.47	23,426

Source: Federal Bureau of Statistics, Government of Pakistan, Islamabad, 2008

The global market for mango fruit demands better shelf-life, uniform size and minimum prices. The leading mango exporting countries have consolidated their positions in the world market during past 10 years as their export volumes have increased by several folds. The export volume of Brazil and Peru has now increased by about twelve- and fourteen-fold, respectively, as compared to 1991. Introduction of efficient packaging houses, more reliable cold chain and changes in mode of export are the main reasons for these increase in export (Anon, 2005).

Since size, colour and appearance of mango fruit influence consumer demand, the maintenance of these attributes throughout the supply chain is critical. Mango fruits between 400 to 600 g fetch a good price in the European market whilst smaller and larger mango fruits are also exported but attain lower prices. Sweeter mango fruits are generally preferred by many consumers in South-East Asia. Conversely, less sweet and less fibrous fruits are generally chosen by people from Europe (Anon, 2005). Mango fruit does not normally need any postharvest treatment when destined for the local market. However, for the global market, fruits are generally harvested early in the season (half-mature stage) to capture market demand. Such fruits are commonly treated with hot water (55^{o}C for 5 minutes) and Etherel in order to enhance uniform ripening. Etherel accelerate the respiration rate and ripening process of mango fruit. Mature fruits can also be ripened with lower doses of Etherel for uniform colour (Rathore, 2007).

2.1.10 Mango trade in Sri Lanka

Mango trees in the tropics have phonological patterns of growth habit, therefore the trees vary significantly from one tree to another. The intermittent dry and wet seasons produce fruits with varying maturity in the same plot and even on the same tree. In general, the selective picking of fruits is not permitted; in contrast the entire fruits from a tree are picked at once. Therefore, ripening and sorting of mango fruit becomes very important (Kulatunga, 1995). Due to these said constraints, mango cultivation in Sri Lanka remains as a small scale operation and caters mainly to the domestic market (Kulatunga, 1995). Inadequate transportation links and improper handling of mango fruits are the major causes for the failure in meeting the demand of the urban and foreign markets. However, a considerable quantity of mango fruit was exported from Sri

Lanka during 2000 and 2001. Yet, no further information is readily available regarding mango export or import from Sri Lanka since this time. Export was about two-fold higher in 2001 than 2000. Export destination and type of product (cut-fresh mango, fresh mango fruit or mango product) was not clearly mentioned but it is assumed that Maldives, Middle East and Europe might be the importing countries. Small quantities of mango products including pickles and chips were imported during 2000 and 2001 from India and China (Table 2.3) (Bogahawatta, 2002).

Table 2.3. Mango production, export and import in Sri Lanka during 2000 and 2001

Mango fruit		2000	2001
Extent (ha.)		25780	25728
Production ('000 fruits)		431047	458987
Export	Volume (kg)	21005	38562
	Value (Rs. Million)	4.61	8.32
Import	Volume (kg)	89.70	117.20
	Value (Rs. Million)	2.00	4.60

Source: Department of Census & Statistics, Sri Lanka

Sri Lankan mango cultivars have more or less similar nutritional and quality characteristics of mango fruits produced from other tropical countries. Indian and Pakistani mango fruits achieve good demand levels and are marketed globally to some extend based on their colour, flavour and nutritional properties. Since the nutritional and quality parameters of tropical mango fruits are higher than most of the commercial mango cultivars, the trade of Indian and Pakistani mango fruits have increased in recent years. However, unlike for India and Pakistan, Sri Lankan mango fruits have not really been exported to date as there are continuing challenges in adopting appropriate postharvest ripening and storage facilities for the long term transport.

The steady and continued growth of export market of quality mango fruits and its products is vital for the development of the Sri Lanka mango industry. However, the overall marketing system of the mango industry is not developed in Sri Lanka. The seasonal rise and fall in production levels, wide price variation, distress sale at throwaway prices during bumper harvest and unsatisfied consumer demand are the major causes for the unsteady market of mango fruits. The commission agents and large traders within the private sectors play a major part in the mango trade in Sri Lanka. The marketing chain begins at the village-level collecting agents, whereas the farmer-assembler-wholesaler-retailer-consumer system is the most common marketing system, more middle men may be involved in different channels based on the distance of the market to the producing area (Kudagamage, 1998).

Mangoes are mainly transported by the open truck (in gunnies and baskets) from the farm to the market or storage, which results in heavy postharvest losses due to the physical damage and dehydration. About 35 to 40% loss was estimated up to the point of consumption due to the lack of knowledge regarding pre- and postharvest handling (Kudagamage, 1998). However, Wijeratnam (1991) reported that postharvest losses are estimated as over 30% during collection and transport of mango fruits due to poor roads, poor transport links and inadequate temperature control facilities.

Mango fruits should be handled with care when destined for the export market as several factors (harvest maturity, treatments to arrest deterioration, pre-cooling and refrigerated transport) affect the final quality. Mango producers must be updated with market information in order to improve the marketing system as delays in marketing mango fruits may result in deterioration in quality and quantity. As far as Sri Lanka is

concerned, the primary producers generally receive market information through the village level traders and fellow producers (Kudagamage, 1998).

Since the local mango cultivars in Sri Lanka are not popular internationally, cvs. Tommy Atkins and Keitt have been introduced to Sri Lanka for export purposes. The farmers who are not equipped with cold storage facilities to handle the produce act as suppliers to exporters. Cut-fruits are often dehydrated and stored as an alternative method of preserving the excess fruit during seasons. However, the product caters to a different market from the fresh fruit market. The seasonal mango fruit production and small scale operation prevent the suppliers in undertaking large investments in postharvest management unless the technology has wide application. The handling of harvested mangoes in Sri Lanka has commonly been blamed for much of the postharvest deterioration before reaching the market place (Kulatunga, 1995).

2.2 Biochemical profile of mango fruit

Though biochemical profiles of mango fruits have extensively been studied globally, there is a paucity of published information on biochemical profile of mango cultivars endemic to Sri Lanka. Several reasons can be suggested for this lack of information; however lack of quality research on Sri Lankan mango fruits using sophisticated instruments is the prime reason.

2.2.1 Non-structural carbohydrates

2.2.1.1 Starch

Starch is a major constituent in mature pre-climacteric mangoes, but starch concentration is very low in ripe mango fruits as it is hydrolysed into sugars *viz.* fructose, glucose and sucrose during ripening (Matto *et al.,* 1975; Subramaniyam *et al.,* 1976; Fuchs *et al.,* 1980; Selvarajah *et al.,* 1989; Lima *et al.,* 2001). However, some findings have shown that there is no more starch in ripe mangoes (Medlicott *et al.,* 1986; Parikh *et al.,* 1990). Starch content usually increases in mangoes during maturation (Saranwong *et al.,* 2004) and the starch is predominant in pulp of unripe mango cv. Tommy Atkins (Kaur *et al*., 2004; Bello-Perez *et al*., 2005). Starch concentration of 46.31% DW of mango cv. Mahajanaka at 105 DAFS in the pulp increased to 55.17% DW at its fully mature stage of 140 DAFS (Appendix C). Accumulation of sufficient amounts of starch at mature stage may allow ripe fruit to synthesis a greater amount of sugar (Saranwong *et al.*, 2004). Breakdown of starch and enzymes activities (sugar synthases) have been observed during mango ripening (Kalra and Tandon, 1983; Ueda *et al.,* 2000). Climacteric rise (rise in ethylene concentration) in mango fruit is indicated by a noticeable increase in amylase activity and sugar content whilst a concomitant decrease in starch content is observed (Lima *et al.,* 2001).

2.2.1.2 Sugars

Sucrose, glucose and fructose are the major sugars in ripe mango fruit, with sucrose contributing to more than 80% in total measured sugar concentration (Hymavathi and Khader, 2005). Total sugar concentration in ripe mango cv. Haden is

roughly 50 to 120 mg g^{-1} FW, whereas sucrose contributes about 74.12% of total measured sugars followed by fructose (20.60%) and glucose (5.31%) (Rathore, 2007). Ripe mango cv. Carabao is reported as having comparatively higher concentration of total sugar whilst very lower sugar content is reported in mango cv. Golek. Sugars constitute about 91% of the soluble solids from mesocarp of the ripe mango cv. Ngowe (Brinson *et al.,* 1988). Fructose is the predominant reducing sugar found in most of the mango cultivars and exhibits a five-fold increase during ripening (Selvaraj *et al.,* 1989). However, sucrose increases in the later stages of mango ripening and this is consistent with the improved activity of gluconeogenic enzyme in the ripe fruit of several mango cultivars (Selvaraj *et al.,* 1989; Fuchs *et al.,* 1980). Mango ripening is stimulated by the increase of gluconeogenic enzymes i.e. glucose-6-phosphatase up to three quarter ripe stage whilst fructose-1, 6-diphosphatase increases from three quarter to fully mature stage (Selvaraj and Kumar, 1995).

Total and non-reducing sugars of ripe mango cv. Delta R2E2 are higher (20.88% and 9.51%, respectively) in fruits ripened at room temperature (24 days) than CA whilst reducing sugars are higher in fruits ripened at CA (Lalel *et al.,* 2005). However, naturally ripened mango cv. Kensington Pride contains higher total sugar content (15.90% DW) including 11.61% non-reducing sugars compare to polyamine treated mango fruits where the total measured sugars vary between 13% to 15.61 % DW (Malik and Singh, 2006) (Table 2.4).

Table 2.4. Sugar concentration of ripe mango pulp from different mango cultivar

Mango Cultivars	Sugars mg g^{-1}				References
	Sucrose	Glucose	Fructose	Total measured sugars	
Haden (FW)	110.0	5.0	44.0	159.0	Castrillo *et al*., 1992
Chiin Hwang 1 (FW)	120.0	16.0	30.0	166.0	Ueda *et al*., 2001
Mahajanaka (FW)	30.9	1.6	31.1	63.6	Saranwong *et al.,* 2004*
Baneshan (DW)	306.9	9.0	45.9	361.0	Hymavathi and Vijaya Khader, 2005
Suvarnarekha (DW)	319.5	10.0	33.8	363.3	Hymavathi and Vijaya Khader, 2005
R2E2 (DW)	113.7		95.1	208.8	Lalel *et al*, 2005
Kensington Pride (FW)	116.0		43.0	159.0	Malik and Singh, 2006
Alphonso (FW)	50.0	39.0	49.0	138.0	Yashoda *et al.,* 2006
Keitt (FW)	87.0	1.0	28.0	116.0	Gonzalez-Aguilar *et al*., 2008
Ataulfo (FW)	120.0	6.0	18.0	144.0	Gonzalez-Aguilar *et al*., 2008
Kent (FW)	70.0	5.0	30.0	105.0	Gonzalez-Aguilar *et al*., 2008
Cat Hoa Loc (FW)	119.4		22.1	141.5	Hoa and Ducamp, 2008
SB Chaunsa (DW)	537.6	9.9	75.0	621.3	Amin *et al*., 2009
Fiaz Kareem (DW)	511.6	5.5	61.9	579.0	Rajwana *et al*., 2010
Chaunsa (DW)	593.0	2.1	22.9	618.0	Rajwana *et al*., 2010
Anwar Rotale (DW)	586.0	30.2	99.7	715.9	Rajwana *et al*., 2010

**Sugars extracted using water (Appendix C)*

2.2.2 Structural carbohydrates

Mango fruit pulp usually becomes soft due to the degradation of structural carbohydrates during ripening. Mango ripening is accompanied by a gradual softening of fruit because of a progressive de-polymerization of pectin and hemicellulose polysaccharides (Yashoda *et al.,* 2006). Textural softening during ripening and storage of mango fruit is crucial as these directly affect the shelf-life and quality of fruits (Yashoda *et al.,* 2007). Depletion of cell wall increases at the climacteric stage during mango ripening due to the disassembly, de-polymerization and dissolution of pectin and other hemicellulose polysaccharides (Fry, 1995). Yashoda *et al.* (2007) and Lazan *et al.* (1986) reported that rapid synthesis of polygalacturonase (PG) activity coincides with

considerable textural alterations during fruit ripening. In that sense, PG enhances the degradation of polyuronide network in fruits and thus causes fruit softening. Carbohydrates are the major fraction in unripe mango fruits, whereas starch and other polysaccharides (dietary fibre) are prominent (Vergara-Valencia *et al.,* 2007). The dietary fibre (DF) mainly consists of celluloses, hemicelluloses, lignin, β-glucans and gums (Figureola *et al.,* 2005; Gallaher and Schneeman, 2001). Total carbohydrate content ranges from 20.81% to 28.20% in mango peel.

2.2.3 Phenolic compounds

Mango fruit is considered as a rich source of dietary antioxidants since it contains phenolic compounds in considerable quantity (Schieber *et al.,* 2000). Fruits and vegetables are rich sources of dietary antioxidents that reduces the risk of chronic diseases in human. Free radicals are a product of tissue metabolism and have the ability to cause potential damage to metabolically active tissue cells. The said damage can be minimised and repaired by the antioxidant capacity and repair mechanism within the cell. Quercetin (Q) and kaempferol (K) glycosides have been identified in mango fruit puree concentrate using HPLC technique coupled with MS detection. Quercetin 3-galactoside (22.11 μg g^{-1} FW), Q 3-glucoside (16 μg g^{-1} FW) and Q 3-arabinoside (5 μg g^{-1} FW) are the prominent flavonol glycosides found in mango (Schieber *et al.,* 2000). Mango peel, a major by-product in the mango industry contributes about 20% of the whole fruit, both unripe and ripe peel are rich sources of valuable components such as polyphenols, carotenoids, dietary fibre, enzymes and other bioactive components beneficial to human health (Wolfe *et al.,* 2003; Ajila *et al.,* 2007b). Polyphenol content ranges from 55 to 110 mg g^{-1} DW in the acetone extract of both unripe and ripe peel of

mango cvs. Raspuri and Badami. Mango peel is specifically rich in phenolic compounds (flavonol o- and xanthone c-glycosides) and pectin with a high esterification (Berardini *et al.,* 2005). A combined recovery of pectin and phenolic compounds from mango peel is successful in diluted sulphuric acid extraction. A total of 129.41 mg g^{-1} DW of polyphenols was detected in the lyophilized dried peel of mango cv. Tommy Atkins, however only 71 mg g^{-1} DW could be detected in the follow up process (Ali *et al.,* 2004).

Gallic acid (6.92 µg g^{-1} FW) is predominant among the phenolic acids, but m-digallic acid, gallotanin, mangiferin and unknown hydrolysable tannins have also been identified in the pulp of mango cvs. Alphonso, Kitchener and Abu Samaka using two dimensional paper chromatography (Saeed *et al*., 1976). However, the presence of flavonols, condensed tannins, chlorogenic and caffeic acids was not confirmed (Saeed *et al*., 1976). Gallic and ellagic acids are the substrates of polyphenol oxydase in mango pulp and peel, respectively (Prabha and Patwardhan, 1986). Mangiferin (1.19 mg g^{-1}) is the prominent flavonoid and contributes about 84% of the total polyphenolics (1.42 mg g^{-1}), whereas isomangiferin (0.05 mg g^{-1}) and mangiferin gallate (0.10 mg g^{-1}) are also found in mango peel. Polyphenolic compounds such as tannic acid (2.72 mg g^{-1}), gallic acid (2.86 mg g^{-1}), ferulic acid (3.30 mg g^{-1}), vanillic acid (3.14 mg g^{-1}) and chlorogenic acid (4.16 mg g^{-1}) are found as prominent compounds regardless of maturity stages (Berardini *et al*., 2005). According to Zhao *et al.* (2006) the levels of phenolic compounds in mature green mango cv. Wacheng gradually decreases during storage, however this can be inhibited by cold-shock treatment, where phenolic compounds in the fruits treated with cold-shock was 66% higher than control fruits on day 12 of storage. Decrease in phenolic compounds towards ripening is positively correlated with

the increase in taste, therefore ripe fruits are preferred by not only human but also pest, rodents and birds than unripe fruits. Higher phenolic content in pre-harvest unripe (green) mango than ripe fruit help them to protect from pest, rodent and bird attack. However, higher phenolic content of peel than pulp in ripe mango fruit may help to reduce pest attach during storage.

Polyphenoloxidase (PPO) generally increases during ripening of mango cvs. Malgoa and Harumanins (Lazan *et al.,* 1986). Mango peel contains higher TP than pulp throughout fruit development (Lakshiminarayana *et al.,* 1970). Kondo *et al.* (2005) reported that total phenolics increase during maturation of mango cv. Choke Anan irrespective of its peel and pulp from 14 DAFB to 56 DAFB. Out of six different unripe and ripe mango cultivars (Deshi, Langra, Deshahari, Chausa, Amrapali and Mallika), total phenolics are higher in ripe fruits of Deshahari, Deshi and Mallika; however a lower concentration of total phenolics is estimated in unripe mangoes than ripe fruits (Singh *et al.,* 2004).

Prusky (1991) reported that the major component of latex in freshly harvested mango cv. Alphonso is 5-[2(Z)-heptadecenyl] resorcinol. Alkenylresorcinols have also been identified in the peel and pulp of freshly harvested mangoes, which is considered as the basis for resistance to some fungal pathogens. Astringency remains perceptible in the mango cv. Carabao until the fully ripe stage and the progressive loss is associated with the loss in total phenolic content.

2.2.4 Non-volatile organic acids

Total measured organic acid (major contribution of citric acid) content of mango fruit decreases significantly during ripening whilst oxalic, tartaric and malic acids show

irregular variations. Percentage loss of citric acid increases during ripening whilst concentration of ascorbic and malic acids increases slightly (Modi and Reddy, 1967; Medlicott and Thompson, 1985). Citric and malic acids are the main organic acids in mango cv. Keitt, however, tartaric, oxalic and ascorbic acids (AsA) are also present in considerable concentrations (Medlicott and Thompson, 1985). Shashirekha and Patwardhan (1976) reported that citric acid is the major organic acid in mango cv. Badami and decreases by about ten-fold during ripening with a slight increase of malic acid.

Ascorbic acid is a water soluble vitamin found predominantly in fruits and vegetables. L-AsA is reversibly oxidised in to L-dehydroascorbic acid (DHA). Both AsA and DHA are biologically active forms and represent the total vitamin C. Further oxidation of AsA may generate diketogulonic acid (no biological function) (Davey *et al*., 2000; Deutsch, 2000). Since AsA is unstable, there may be some difficulties in extraction and quantification. UV-visible spectrophotometric, enzymatic, electrochemical and florimetric techniques are the most common methods followed to determine AsA and DHA, but these techniques show a very poor sensitivity and selectivity for DHA (Pastore *et al*., 2001).

Both peel and pulp of mango fruit are good source of AsA. However, the concentration varies extensively based on cultivar, tissues, stage of maturity, postharvest ripening and storage, climatic conditions, cultural practices and pre- and postharvest factors (Lee and Kader, 2000). Mango peel has the potential to be used in the food system, but no reported evidence is found yet. Vinci *et al*. (1995) reported that AsA concentration of mango pulp ranges from 0.25 to 1.83 mg g^{-1} FW (cvs. Keitt, 0.33 mg g^{-1}; Kent, 0.32 mg g^{-1}; Tommy Atkins, 0.26 mg g^{-1}; Mansour *et al.*, 2006).

However, cvs. Langra and Haden (Tovar *et al*., 2001) have relatively higher content of AsA than said cultivars. There is an increase in AsA concentration in mango cv. Choke Anan peel from 14 to 84 days after full bloom (DAFB) whilst decreases in pulp (Kondo *et al.,* 2005). Ascorbic acid is considered as an important antioxidant in human nutrition and act as an enzymatic co-factor (Cruz-Hernandez *et al*., 1997).

Titratable acidity of mature mango cv. Ataulfo reduces during storage under different conditions (temperature and ethylene) in refrigerated and non-refrigerated conditions (Montalvo *et al.,* 2007). However, no significant variation is observed in titratable acidity of mango cvs. Keitt, Kent and Tommy Atkins studied in different hot air and hot water treatments (Mansour *et al.*, 2006). Decrease of acidity during ripening might be due to citric acid degradation, conversion in to sugars and further utilization in metabolic process in fruits (Rathore *et al*., 2007).

2.2.5 Sugar/acid ratio

Taste is the balance between sugar and acid (Malundo *et al*., 2001), such that increasing sugar/acid ratio during ripening generally improves perceived taste. The sweetness of sugars and sourness of organic acids are responsible for the taste of many fruits (Kays, 1997). Sri Lankan mango cvs. Karutha Colomban and Willard have excellent sweetness with relatively high Total Soluble Solids (TSS)/acid (131.91 to 165.30) ratio. Whereas mango cv. Willard has higher TSS, pH and total titratable acidity than other cultivars, but titratable acidity reduces during ripening (7 days ripening at ambient condition of 32^{o}C and 65% RH) whilst TSS and pH increase (Krishnapillai, 2004). It is supported by Mizrach *et al.* (1997) that carbohydrate and acid metabolism are closely associated during postharvest ripening as acidity decreases more

rapidly than sugars increase. A randomly selected taste panel (different range of people) was used to assess and appraise the ripe pulp of cv. Dosehari; according to the response of the taste panel the score increases from 5 to 8.46 at day 3 and then decreases to 1.67 at the end of day 15 during ripening at 32^{o}C to 35^{o}C (Rathore *et al.*, 2007). The variation in taste score might be due to the fluctuations in acids, pH, sugars and sugar/acid ratio. Sugars and acids are the primary taste compounds which enhance human perception of taste (Malundo *et al*., 2001).

Total sugar (alcohol soluble) of unripe and ripe mango fruits are correlated with organic acids, starch and cell wall constituents such as cellulose, hemicellulose and pectin. The glucose, fructose and sucrose of unripe mango fruit (3.81, 6.10 and 0.22 mg g^{-1}, respectively) increase by several folds during ripening (39, 49 and 5 mg g^{-1}, respectively) whilst citric and succinic acids of unripe fruit (24.81 and 2.90 mg g^{-1}, respectively) decrease by about seven-fold (2.21 and 0.31 mg g^{-1}, respectively). However, malic acid increases from 0.32 mg g^{-1} to 1.60 mg g^{-1} in ripe fruits. Starch (18% to 0.11%), cellulose (2% to 0.90%), hemicellulose (0.80% to 0.22%) and pectin (1.91% to 0.50%) are in decreasing trend during ripening (Yashoda *et al.,* 2006).

2.2.6 Aroma volatiles

The flavour of mango induces consumers to buy more fruits, which in-turn would be expected to increase market demand (Gholap *et al.*, 1986). A number of volatile compounds have been identified in mango fruit, yet mango cultivars differ widely based on the type and concentration of volatile compounds are present (especially depending on the location/agronomy). Mango cultivars native to tropical countries generally contain more oxygenated compounds (esters, furanones and

lactones) whilst mango cultivars grown in rest of the world are mainly the hybrids of Asian mango stock, which is rich in certain hydrocarbons (3-carene) (MacLeod and de Troconis, 1982; Wilson *et al*., 1990; Narain *et al*., 1998). Since the characteristic flavour of mango varies from cultivar to cultivar, the flavour is used as one of the factors to differentiate cultivars. MacLeod and Pieris (1984) demonstrated that the distinct differences among cultivars can be attributed to volatile compounds unique to each cultivar. For example, (Z)-3-hexenyl esters are responsible for the fresh, green fruity flavour of mango cv. Alphonso whilst C9-lipid oxidation product, (E)-2-nonenal is accountable for the melon-like flavour in cv. Baladi. Volatile compounds of fruits are estimated using different instruments like GC-MS, GC-FID, GC-EIMS and solvent extraction, however incorporating sensory panel with the findings is recommended to differentiate cultivars based on the aroma.

Several volatile components including ethanol, acetic acid, amyl acetate, 2-phenyl ethanol and phenyl ethanol acetate have been identified in the tenax extracts of over-ripe mango fruits using mass spectral and retention index comparisons with synthetic standards (Zhu *et al.,* 2003). Selvaraj (1989) pointed out that (Z)-ocimene has been identified as the principal volatile constituent of mango cv. Alphonso as it has a very strong aroma among the Indian mango cultivars. Characteristic mango flavour could not be attributed to any specific component. However, *cis*-ocimene and β-myrcene may be responsible for the green aroma of unripe mango fruit whilst dimethylstyrene has a mango-like character (Engel and Tressl, 1983; MacLeod and Pieris, 1984). A number of volatile compounds (glycosidically-bound) have been identified in peel and pulp of mango cv. Kensington Pride at different maturity stages (mature green, half mature and ripe). Mature green peel and pulp of mango fruit contain

trace amounts of volatile compounds (*cis*-3-Hexanol and Hexadecanoic acid); however volatiles increase during ripening (ethyl-9-hexadecanoate, ethyl (Z)-9-octadecanoate, ethyl hexadecanoate methyl dihydromalvalate and λ-undecalactone). It has also been observed that volatile compounds are strongly influenced by the spatial and temporal variations of fruits (Lalel *et al.,* 2003a). The concentration of volatile compounds varies between peel and pulp, and also over the ripening period of mango fruit. Pino and Mesa (2006) concludes that ethyl-2-methylpropanoate, ethyl butanoate, (*E,Z*)-2,6-nonadienal, (*E*)-2-nonenal, methyl benzoate, *(E)-β*-ionone, decanal and 2,5-dimethyl-4-methoxy-3(2H)-furanone are the important components responsible for the aroma of mangoes. Numerous other volatile components are also present in mangoes, however they do not make any significant impact on mango aroma.

Terpinolene is the prominent volatile compound in Sri Lankan mango cvs. Willard (135.5 μg g^{-1} FW) and Parrot (219.81 μg g^{-1} FW) whilst Ocimene (95.1 μg g^{-1} FW) dominate the volatile composition in cv. Karutha Colomban. However, the volatile compounds such as myrcene, 3-carene, α-pinene, β-pinene, limonene, α-caryophyllene and α-humulene are also present in considerable quantities (Table 2.5). The said volatiles were extracted using a modified Likens and Nickerson apparatus and analysed using GC-EIMS and GC-CIMS along with GC-MS and GC-FID (MacLeod and Pieris, 1984). Mango cvs. Kensington Pride and Delta R2E2 are rich in terpinolene (2815 μg g^{-1} FW: Lalel *et al*., 2003b and 944.12 μg g^{-1} FW: Lalel *et al*., 2003b, respectively) whilst 3-carene is the main volatile in cv. Kent (401 μg g^{-1} FW: Torres *et al*., 2007).

Scientists have attempted to identify and quantify volatile compounds of mango fruit using different methods. Steam distillation and/or solvent extraction procedures were employed in the extraction of volatiles in earlier studies (MacLeod and Snyder,

1985; Idstein and Schreier, 1985; Gholap *et al.*, 1986), but these methods are believed to modify the flavour profile of a fruit sample quantitatively or qualitatively (Ackerman and Torline, 1984; Sakho *et al.*, 1985; Bartley and Schwede, 1987). Moreover, steam distillation and/or solvent extraction techniques are not considered appropriate for large number of samples, such that a headspace sampling method could be used, which involves trapping volatile components over a period of time on a solid support. Solid Phase Micro Extraction (SPME) fibre is one of the most efficient solid supports, which is a solvent-free, rapid and sensitive technique and has become popular since it has been successfully used for qualitative and quantitative analysis of volatiles in fruits (Lalel *et al*., 2003a). The solid support is subsequently inserted into the GC injection system and thus allows the volatile compounds to be carried into a GC column by a carrier gas (Sakho *et al.*, 1985; Bartley and Schwede, 1987; Koulibaly *et al.*, 1992). Bartley and Schwede (1987) suggested that a headspace chromatogram reflects more closely the true flavour profile of a mango sample compared to one generated by distillation and solvent extraction, but compounds present at a lower concentration may not be detected effectively using headspace technique. However, SPME technique coupled with GC is recommended over all other existing techniques to extract and quantify aroma volatile compounds effectively from fruits.

Table 2.5. Concentration of volatile compounds of ripe mango fruit pulp from different cultivars

Mango Cultivars	Volatile compounds µg kg^{-1} FW											Reference
	Toluene	α-pinene	β-pinene	Myrcene	3-carene	α-terpinene	Limonene	Ocimene	Terpinolene	β-caryophyllene	α-humulene	
Willard	41.1	27.9	8.9	5.9	11.9	5.9	16.0	4.2	135.5	10.1	5.9	MacLeod and Pieris, 1984
Karutha Colomban	6.8	ND	7.3	10.8	ND	ND	6.5	95.1	ND	11.5	6.5	MacLeod and Pieris, 1984
Parrot	22.6	10.0	17.0	13.8	73.8	24.5	19.5	11.9	219.8	6.3	7.5	MacLeod and Pieris, 1984
D-R2E2	ND	19.3	1.3	19.3	117.3	10.7	15.6	3.7	944.1	ND	ND	Lalel and Singh, 2006
Irwin	ND	700.0	500.0	400.0	7200.0	ND	1100.0	600.0	ND	ND	ND	Quijano *et al*., 2007
Manila	ND	ND	ND	400.0	15100.0	ND	1500.0	600.0	1100.0	ND	ND	Quijano *et al*., 2007
Tommy Atkins	ND	400.0	400.0	400.0	10100.0	100.0	100.0	ND	600.0	ND	ND	Quijano *et al*., 2007
Van Dyke	ND	1900.0	200.0	400.0	100.0	ND	600.0	100.0	800.0	ND	ND	Quijano *et al*., 2007
Kent	ND	10.0	ND	17.0	401.0	ND	13.0	ND	ND	ND	ND	Torres *et al.,* 2007
Kensington Pride	ND	27.5	1.5	32.4	107.0	47.0	23.3	1.0	966.7	47.9	31.0	Dang *et al*., 2008a
Keitt	ND	540.0	850.0	790.0	4920.0	ND	1410.0	860.0	ND	1800.0	1010.0	Pandit *et al*., 2009a

ND-not detected

2.2.7 Pigments

A number of pigments are responsible for the objective colour of mango fruits. Changes in colour during ripening gain more importance since it is responsible for the indication of ripening stage and consumer acceptance. Colour of unripe mango pulp turns to yellow-orange during ripening in most cultivars whilst peel colour (green) turns to yellow-red in some cultivars. The colour change is mainly caused by the degradation of chlorophyll that initially masks the previously present pigments and synthesis of anthocyanins, carotenoids and xanthophylls esters. Fruit colour can non-destructively be measured using colorimeters, spectrophotometers and colour machine vision systems. However, hand-held colorimeters are usually used to measure the surface colour since this is very handy for on-farm colour measurement (Yashoda *et al*., 2006). Colour parameters are measured based on the variation in L* (luminosity), C* (chroma) and h^o (hue angle) values of mango fruit. The h^o value of mango peel usually decreases during ripening whilst L* and C* increases. Decrease in h^o indicates the colour change from greenish to yellow and/or orange, but the increase in L* and C* values improve the colour purity (Tovar *et al*., 2001).

Carotenoids are considered as the prime pigments in ripe mango fruit and are responsible for their vibrant colour (Godoy and Rodriguez-Amaya, 1989; Litz, 2009). Mango is abundant in carotenoids; β-carotene contributes more than half of total carotenoid (48 to 80%) in most mango cultivars. In tropical countries, mango substantially contributes to the β-carotene supply in the human diet (Cano and De Ancos, 1994). Mango fruit (peel and pulp) colour varies based on the concentration and variety of carotenoids (Mercadante *et al.,* 1997; Chen *et al.,* 2004). Beside β-carotene, carotenoids such as violaxanthin, mutaloxanthin and luteoxanthin have also been found

in different Brazilian mango cultivars (Godoy and Rodriguez-Amaya, 1989). In addition to all-*trans*-β-carotene, mango cv. Keitt contains all-*trans*-violaxanthin and 9-*cis*-violaxanthin also as principal carotenoids. Carotenoids composition varies based on the geographic or climacteric effects, stage of maturity, cultivar, fruit processing, and storage condition during shipment (Mercadante and Rodriguez-Amaya, 1998). The β-carotene content varies widely among mango cultivars i.e cv. Malgoa (8 μg g^{-1} FW) contains about fifteen-fold lower content than cv. Alphonso (130 μg g^{-1} FW) (Hymavathi and Khader, 2004). John *et al*., (1970) reported that phytofluene is the main carotenoid in ripe mango fruits whereas β-carotene is the major carotene in unripe mango.

Anthocyanidin and peonidin-3-galactoside are commonly responsible for the red blush colour of cv. Haden (Anon, 2009a). Total carotenoids concentration in ripe mango fruit usually ranges between 9 to 92 μg g^{-1} FW. However, Indian mango cv. Alphonso is reported to have higher content of total carotenoids (110 μg g^{-1} FW) (Padmini and Parabha, 1997; Litz, 2009) than other cultivars. The total carotenoids and β-carotene contents of mango cv. Ataulfo are around 12 μg g^{-1} FW and 5 μg g^{-1} FW, respectively (Corral-Aguayo *et al*., 2008). Miller *et al*. (1996) reported that among the various carotenoids present in mango fruits, lycopene has higher antioxidant activity followed by β-caryophyllene, β-carotene, lutein and zeaxnthin. About 25 carotenoids have been identified from a pooled sample of 40 Taiwanese mango cultivars (ripe pulp) using an HPLC method, whereas all-*trans*-β-carotene is prominent (29.31 μg g^{-1} FW) followed by *cis* isomers of β-carotene (9.86 μg g^{-1} FW), violoxanthin and its *cis* isomers (6.40 μg g^{-1} FW), neochrome (5.03 μg g^{-1}), luteoxanthin (3.61 μg g^{-1} FW), neoxanthin and its *cis* isomers (1.88 μg g^{-1} FW), zeaxanthin (1.16 μg g^{-1} FW) and 9- or 9'-cis-lutein (0.78 μg

g^{-1} FW) (Chen *et al.,* 2004). Malik and Singh (2006) reported that mango cv. Kensington Pride contains about 21 μg g^{-1} FW of total carotenoids. An unusual carotenoid (violaxanthin dibutyrate) was identified in mango cv. Kent along with other carotenoids such as 9-*cis*-violaxanthin and neochrome (luteoxanthin) (Pott *et al*., 2003).

Carotenoids were separated from mango using a reverse phase HPLC coupled with a C-18 column or a normal phase HPLC comprises a nitrile column, However, Emenhiser *et al.* (1995) has proved that C-30 column provided better resolution of geometrical isomers of carotenoids than C-18 column (Cano and de Ancos, 1994; Mercadante *et al.,* 1997). Total carotenoids can be extracted using alcoholic potassium hydroxide and separated in petroleum ether and subsequently be analysed using spectrophotometer (Malik and Singh, 2006).

2.3 Chemometric analysis

Chemometrics is defined as the science of extracting information from multivariate data using the frequently employed methods in core data analysis such as multivariate statistics, applied mathematics and computer science. One or two variables have traditionally been analysed at a time from a multivariate data, but all the samples and variables of a multivariate data can be analysed simultaneosly using chemometric analysis. It has usually been performed using principal component analysis (PCA), hierarchical component analysis (HCA), classification and discriminant analysis (CDA) and multiple regression analysis (MRA) on volatile fingerprints/profiles and rarely on data obtained from aqueous chemical profiles (Rudell *et al.,* 2008).

Chemometrics is a highly interfacial discipline, characteristically used for one or more of the primary purposes *viz.* exploring patterns of association in data, tracking

properties of materials on a continuous basis and preparing and using the multivariate classification models. Patterns of association exist in many datasets, however it becomes difficult to discover the relationship among samples when the data matrix exceeds three or more features. Even hidden patterns in a complex data can be revealed effectively using the exploratory data analysis by reducing the information to a more comprehensible form. The large complex data sets can be reduced in to a series of optimised interpretable views using the exploratory algorithms such as PCA and HCA. The interpretable views highlight the natural groupings in the data and point out the strong influence of variables on these patterns. The PCA and HCA are 'unsupervised' approaches, meaning that no prior knowledge of the sample types is used in the analysis. Intrinsic variance between the samples without being biased towards desired outcomes is the prime benefit of 'unsupervised' chemometric approaches (Thanaraj *et al*., 2009).

2.3.1 Principal component analysis (PCA)

Principal component analysis can mathematically be defined as an orthogonal linear transformation of data to a new coordinate system. Therefore, PCA transforms the data in least square terms. PCA is a powerful and useful statistical technique for finding patterns in the multivariate data and expressing the data in such a way to highlight their similarities and differences. The spectral data is compressed in to a set of uncorrelated orthogonal factors (principal components (PCs)) by a series of rotation and projections. The PCs are computed from the matrix of correlations between the variables. The first PC accounts for as much of the variability in the data as possible and each succeeding component accounts for the remaining variability. These PCs are ultimately used to

summarise the original data, thus reducing the variables to a smaller number without altering the available information (Daschner *et al.,* 2000). PCA is able to discriminate between cultivars on the basis of biochemical composition, since cultivars which contain similar concentrations of specific biochemical analytes are clustered together (Gine Bordonaba and Terry, 2008). Adulterated and pure fruit samples are also discriminated using PCA effectively based on colour variation patterns.

The PCA bi-plot is a two or three dimensional plot showing a set of data points and a set of axis. The simplest PCA bi-plot shows first two PCs together with projections of axes of the original variables. Each observation (row of scores) is represented as a point in the bi-plot. The Euclidean distances between the data points on first two PCs represent the Mahalanobis distance and inner products between the connecting lines of these points represents covariance. Mahalanobis distance is based on correlations between variables by which different patterns can be identified and analysed. It accounts for the variance of each variable and the covariance between variables. The normalised mahalanobis distance performs well once the intrinsic dimentionality of the data distribution is known beforehand (Jackson and Chen, 2004). A bi-plot visualises the magnitude and sign of each variable that contributes to the first two or three PCs and subsequent observations represented in terms of those PCs. Bi-plot imposes a sign convention, whereas the elements with largest magnitude is forced in each column as positive whilst some of the vectors are flipped to the opposite direction. Therefore, the plot becomes easier to read without affecting the interpretation since changing the sign of a coefficient vector does not change its meaning.

A text type PCA bi-plot analysis (Figure 2.8) clearly demonstrates the interpretation of data and the extraction of information from a bi-plot analysis (Nishina,

2007). Fifteen texts are divided in to three categories (1: imaginary prose-literature works, 2 and 3: informative prose), whereas category 1 is K, L, M, N, P, R, category 2 is A, F, G, J and category 3 is B, C, D, E, H. The texts A, B and C are news paper press, but text A is clustered in category 3 whilst B and C are grouped in category 2. The J and H are academic texts, but clustered away in category 3 and 2, respectively. Therefore, imaginary prose is clustered away from informative prose on PC1 (majority of the variance) as those are the major discriminatory factors. Categories 2 and 3 have grouped on PC2 (less variance), but there is a crossover of text A between categories 2 and 3.

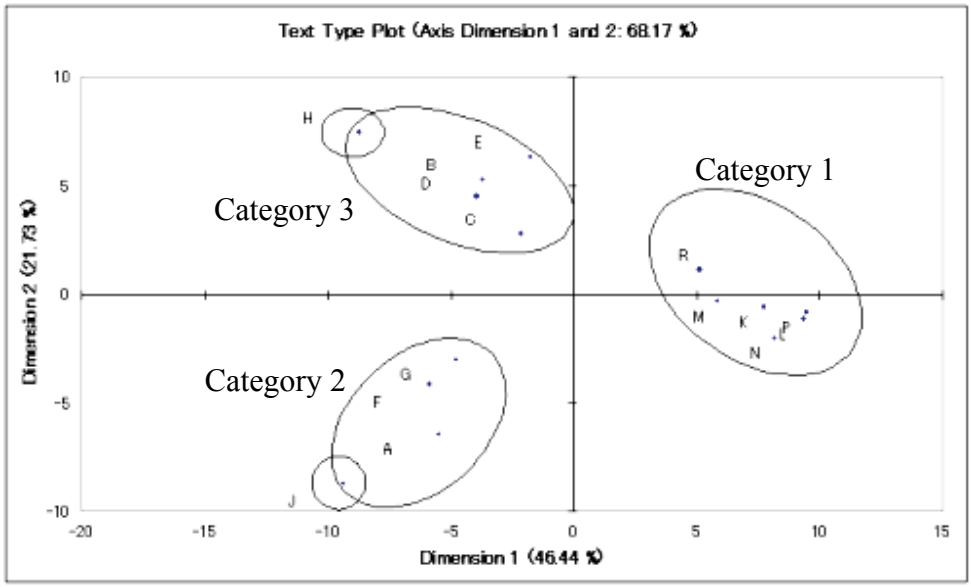


Figure 2.8. Text type bi-plot based on principle components analysis. The lines are added as an aid to locate groups (Nishina, 2007)

Quemener *et al.* (2007) analysed the chromatographic finger print data of tomato cell wall polysaccharides using chemometric analysis. The PCA bi-plot (Figure 2.9) describes the distribution of corrected chromatograms of first two principal components. The first principal component (64.90% of the total variance) rank CER > LCX >

LC9≈LC4 > LEV since the genotype samples C and X are clustered dominantly on PC1. Therefore, genotypes CER and LCX are the major discriminatory factors in this analysis. Furthermore, genotype samples L, 4 and 9 are clustered predominantly in PC2.

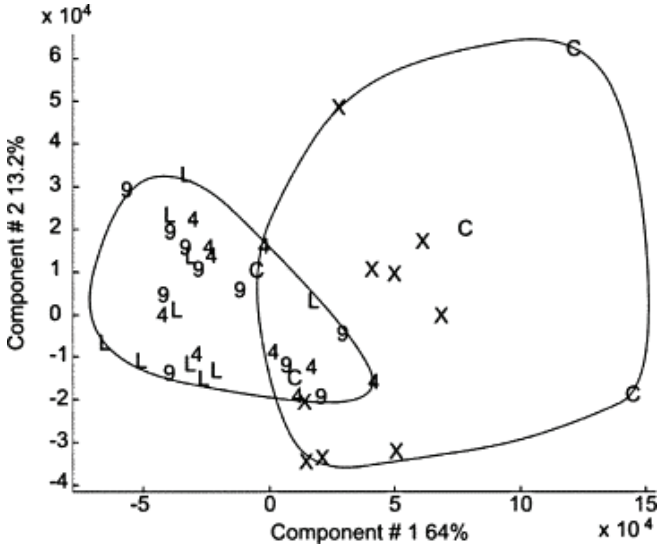


Figure 2.9. Principal component analysis of corrected high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC–PAD) chromatograms (8–30 min region) collection generated by endo-xylanase treatment of alcohol insoluble material prepared from pericarp cell walls of tomato genotypes LC4 (4), LCX (X), LC9 (9), LEV (L) and CER (C) collected in 2002 and 2003. The lines are added as an aid to locate groups (Daschner *et al.,* 2000)

In a separate study, data collected using an electronic tongue (array of metallic potentiometric sensors) from different food stuffs such as vinegars and fruit juices was analysed using PCA (Lachenmeier, 2007). The scores and loading were presented in PCA represents 100% of total system variance with a clear separation of all measured samples. Only samples with measurement errors are removed to obtain robust models covering a comparatively higher variation with a complete sample collection. Two main areas are indicated based on the first two PCs. A preliminary PCA discrimination result

is used to distinguish the groups of juices corresponding to different tests. The juices produced by the same manufacture are united into two separate groups according to the common features of the similar production procedures (Lvova *et al.,* 2002). Essential oils have also been separated from mandarin fruit peel using gas chromatography and chemometric analysis (Controneo and Dugo, 1988).

Chemometric procedures coupled with GC-MS are usually carried out to interpret the aroma profile of plant materials. However, PCA has now been used to analyse aroma profiles eliminating from the HS-SPME data. If partial least square analysis is possible, it can be used to further interpret the aroma profile (Zhang and Li, 2007).

2.3.2 Hierarchical cluster Analysis (HCA)

Cluster analysis (CA) is preliminary used to understand the data sets for obtaining natural groupings among samples containing measured features. Cluster analysis (pattern recognition technique) is performed on auto scaled data and is used to reveal the structure in a dataset due to its unsupervised nature (Massart and Kaufman, 1983). Sample similarities are usually calculated based on squared Euclidean distance and the Ward hierarchical agglomerative methods, which are subsequently used to establish clusters. When CA is applied to the complete set of variables, sometimes no clear groupings of samples may be obtained. However, enough information can be achieved by a classification in established categories by considering only the most distinct variables. HCA shows that biochemical composition of different cultivars is related to parentage information. Cultivars that contain more or less similar ranges of

biochemical concentration are clustered together and are also related to each other by genotypic and geographical factors (Gine Bordonaba and Terry, 2008).

The HCA analysis is also performed on a text type data containing fifteen letters (K, L, M, N, P, R, A, F, G, J, B, C, D, E and H) in three different categories as described previously in order to understand the clustering of samples according the intrinsic variance (Figure 2.10). Clustering of samples is coincided with the grouping of PCA bi-plot of the same dataset (Figure 2.8). Briefly, category 1 is clustered away from categories 2 and 3 and then category 2 and 3 are further clustered (Nishina, 2007). In a separate study, a natural separation is observed between unripe and ripe apple fruits in a HCA analysis, but the clustering differs significantly between peel and pulp based on the degree variance in similarities of both peel and pulp (Figure 2.11) (Alonso-Salces *et al.,* 2005).

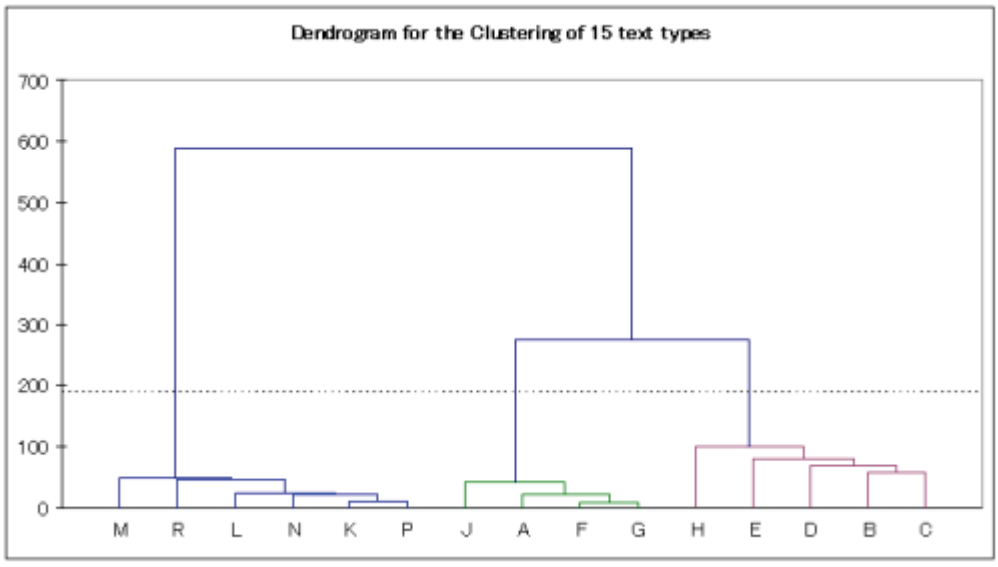


Figure 2.10. Cluster analysis of 15 text types (K, L, M, N, P, R, A, F, G, J, B, C, D, E and H) (Nishina, 2007)

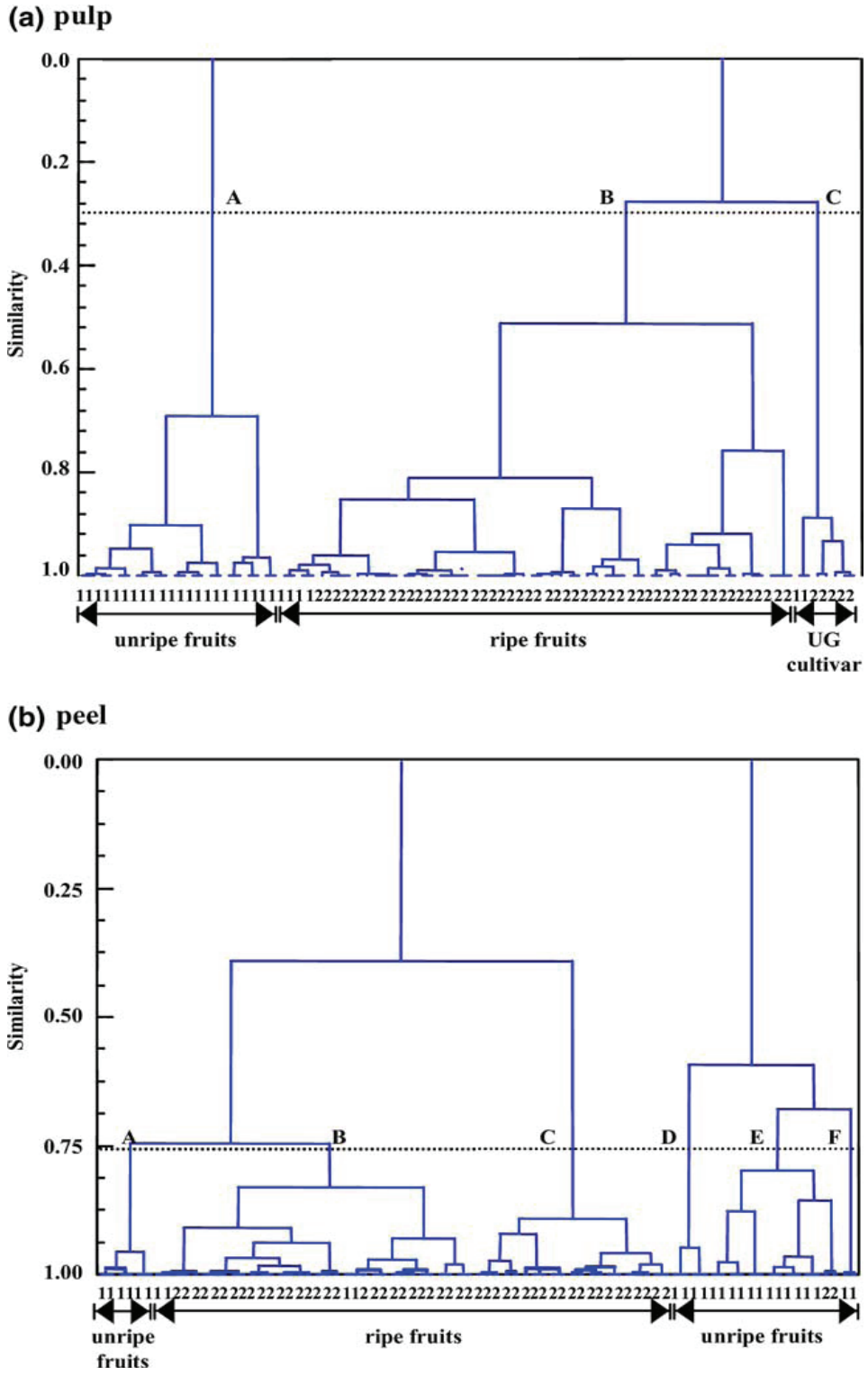


Figure 2.11. Dendrograms of cluster analysis for apple fruit samples. Sample codes: 1-unripe fruits; 2-ripe fruits. Thus, the variables regarded in peel were DPn, PLD-2, PLD-1 and PLXG, and in pulp, DPn, PC and CAT (Alonso-Salces *et al.,* 2005)

2.4 Conclusions

Mangoes are widely distributed in tropics and considered as one of the tropical fruits mainly consumed for aroma and taste. However, mango fruit has also been eaten in many temperate countries. Therefore, defining and elucidating the biochemical composition, quality, packaging, storage and shelf-life of mango fruit is gaining more importance as far as consumer acceptance and market demand are concerned. Several mango cultivars are grown in Sri Lanka; however cvs. Willard and Karutha Colomban are considered as economically important cultivars as they attain higher demand in local markets than other commercial cultivars. Though mango cultivars have been studied extensively around the world, there is a paucity of published information on biochemical profile, shelf-life and quality of Sri Lankan mango cultivars. Pre-climacteric mango fruits are rich in starch whilst ripe mango fruits contain sugars, organic acids, AsA, TP, flavonoids, carotenoids and various volatile compounds. However, mango peel (major by-product in mango industry) is also a rich source in TP and flavonoids. Therefore, eating mango fruit can also provide dietary antioxidants that may improve human health. Mango fruits have been extracted and analysed using several methods to quantify the individual biochemical compounds, but it is noted that extraction and quantification methods influence the final concentration. That said, analysing biochemical compounds using improved techniques may help to optimise the concentration, since these improve extraction and/or detection, separation, analysis and quantification. Chemometric analysis is usually conducted using PCA and HCA; both are unsupervised techniques (meaning that there is no prior knowledge about the samples). The benefit of such approaches is that samples can be clustered without being biased towards the desired outcomes.

CHAPTER THREE

Chemometric analysis of pre-climacteric Sri Lankan mango (*Mangifera indica* L.)

3.1 Introduction

Total mango production in Sri Lanka is around one hundred thousand tonnes per annum. Mangoes are derived from three different agro-ecological regions; dry zone, wet zone and intermediate zone. Unique colour, flavour, sweetness and aroma are distinct properties of mangoes harvested in the dry zone of Sri Lanka, which attain greater consumer demand. However, lower production volumes yet higher pre- and postharvest losses limit processing and export possibilities (FAO, 2005). A seasonal mango harvesting pattern is observed in Sri Lanka as crops bloom from January to March and are harvested in May to July in wet and intermediate zones (Yala season crops). Mangoes from the dry zone bloom between July to September and are harvested from November to January (Maha season crops). Therefore, production drops in between the two seasons resulting in higher prices than in-season (Peiris and Senevirathna, 2001).

Mangoes should be harvested at a physiologically mature stage for subsequent postharvest ripening, however, harvesting criteria vary according to local consumption patterns and distance to market. Fruit maturity at harvest is judged by the shape of fruits, however, days after fruit set (DAFS) is one of the most critical factors affecting subsequent ripening, flavour development and postharvest utilization (Lizada, 1993). According to Kosiyachinda *et al*., 1984, external properties such as number of days after full bloom (DAFB) or DAFS, protrusion of shoulder, peel colour and a degree of 'bloom' on the fruit surface have also been used as predictors of harvest maturity; however these properties have no direct impact on eating quality.

Mature green mangoes attain a superior eating quality when ripe whilst immature ones do not; thus measuring the harvest quality of pre-climacteric mango fruit is very important in order to discriminate immature and mature fruits at harvest (Saranwong *et al*., 2004). The concentration of accumulated starch and dry matter is understood to determine harvest quality (Tandon and Kalra, 1983; Ueda *et al*., 2000). Starch is the major carbohydrate present in mature green mangoes and is hydrolysed into sugars during ripening (Lima *et al*., 2001). Only trace amount of starch and reduced amylase activity can be detected in over-ripe mangoes (Lima *et al*., 2001).

Much research has been undertaken to quantify non-structural carbohydrates (NSCs), organic acids and TP in various pre-climacteric mango cultivars (Table 3.1). However, published information on chemical profiling is very rare for mangoes that are endemic to Sri Lanka. Harvest maturity plays a significant role in the quality and nutritional composition of postharvest mango fruit. Mango should be harvested at appropriate maturity to develop favourable colour, flavour and nutritional properties during subsequent postharvest ripening. Therefore, it was necessary to optimise the harvest maturity of pre-climacteric mango fruits using biochemical profile in combination with currently available methods, the said methods are considered as ineffective when they are used alone or in combination. The aim of this study was to improve the understanding of spatial distribution and temporal changes of chemical composition in pre-climacteric Sri Lankan mangoes using chemometric analysis, which may assist in predicting optimum harvest maturity. Concentration of non-structural carbohydrates, acids, total phenolics and dry matter at harvest could be considered to optimise the harvest maturity since they may be responsible for the final quality of mango fruit.

3.2 Materials and methods

3.2.1 Plant material

Five prominent mango cultivars, endemic to Sri Lanka (*viz*. Willard, Karutha Colomban, Vellai Colomban, Ampalavi and Malgova), were randomly selected and fruits were harvested at three different maturities from different trees (*viz.* immature (I), half mature (M_H) and fully mature (M_F)) from the Eastern University Agronomy Farm (Batticaloa, Sri Lanka). Fruit maturity was based on the DAFB, fruit shape and maturity of seed stone during destructive opening. Samples were prepared from fruits immediately after harvest. Each fruit (n = 9 per cultivar) was divided into nine sections. Vertical transects (VT) were made by cutting each fruit longitudinally using a sharp knife adjacent to the seed coat and a 3 cm wide longitudinal section removed. This section was then equally divided into stem end, middle and distal end pieces. Horizontal transacts (HT) were taken from each section of mango as peel (P), outer pulp (OF) and inner pulp (IF). The average fresh weight of each peel and pulp sample was between the ranges of 5-10 g.

Samples were immediately snap-frozen in liquid nitrogen and then stored briefly at -20°C. Deep-frozen samples were put on dry ice in an insulated expanded polystyrene box before being transported (8 h journey) from Eastern University to Colombo. Upon arrival, samples were then put on fresh dry ice before being air freighted from Sri Lanka to London. Samples remained fully frozen during the 2-day transit. Frozen samples were subsequently freeze-dried in an Edwards Modulyo (W. Sussex, UK) freeze drier, milled to a fine powder before being returned to -20°C until use. Dry matter was calculated as a proportion of FW.

The texture and firmness of mango fruit vary during maturation, however these parameters were not analysed due to the lack of facilities at the Eastern University, Sri Lanka.

Table 3.1. Concentration of sugars, starch, non-volatile organic acids and total phenolics of pre-climacteric mango cultivars

Cultivars	Concentrations	References
	Sugars	
Tommy Atkins	Reducing sugars: 15 mg g^{-1} FW Non reducing sugar: 39 mg g^{-1}	Lima *et al*. (2001) [a]
Mahajanaka	Total measured sugars (FW): 60.06 mg g^{-1} (105DAFS), 63.64 mg g^{-1} (140DAFS) Fructose: 30.03 mg g^{-1} (105DAFS), 31.09 mg g^{-1} (140DAFS) glucose: 2.93 mg g^{-1} (105DAFS), 1.63 mg g^{-1} (140DAFS) Sucrose: 27.10 mg g^{-1} (105DAFS), 30.91 mg g^{-1} (140DAFS)	Saranwong *et al*. (2004)
Delta R2E2	Total measured sugars (DW): 208.82 mg g^{-1}, Fructose + glucose: 95.10 mg g^{-1} Sucrose: 113.71 mg g^{-1}	Lalel *et al*. (2005)
Alphonso	Total measured sugars (FW): 10.10 mg g^{-1}, Fructose: 6.12 mg g^{-1}, Glucose: 3.80 mg g^{-1}, Sucrose: 0.21 mg g^{-1}	Yashoda *et al*. (2006) [a*]
	Starch	
Tommy Atkins	Starch: 11.20% DW	Lima *et al.* (2001)
Mahajanaka	Starch: 46.31% DW (105 DAFS) and 55.17% (140 DAFS)	Saranwong *et al*. (2004)
Tommy Atkins	Starch: 29.88% DW	Vergara-Valencia *et al*. (2007)
	Organic acids	
Choke anan	Ascorbic acid (mg g^{-1} FW): 14 DAFB:0.05 (peel), 0.19 (pulp) 56 DAFB:0.10 (peel), 0.12 (pulp) 84 DAFB:0.14 (peel), 0.13 (pulp)	Kondo *et al*. (2005)
Raspuri	Ascorbic acid (DW): 0.19 mg g^{-1}	Ajila *et al.* (2007a)
Badami	Ascorbic acid (DW): 0.32 mg g^{-1}	Ajila *et al.* (2007a)
Alphonso	Citric acid (DW): 24.81 mg g^{-1}, Malic acid (DW): 0.30 mg g^{-1} Succinic acid (DW): 2.91 mg g^{-1}	Yashoda *et al.* (2006)
	Total phenolics	
Tommy Atkins	Total extractable phenolics (DW): 16.14 mg g^{-1}	Vergara-Valencia *et al*. (2007)
Raspuri	TP (DW): 29.41 mg g^{-1} (buffer extract), 73.88 mg g^{-1} (alcohol extract), 109.70 mg g^{-1} (acetone extract)	Ajila *et al*. (2007a)

Badami	TP (DW): 20.11 mg g^{-1} (buffer extract), 37.92 mg g^{-1} (alcohol extract), 90.18 mg g^{-1} (acetone extract)	Ajila *et al*. (2007a)
Choke Anan	14 DAFB:0.09 mg g^{-1} FW (peel), 0.06 mg g^{-1} FW (flesh) 56 DAFB:0.23 mg g^{-1} FW (peel), 0.15 mg g^{-1} FW (flesh) 84 DAFB:0.20 mg g^{-1} FW (peel), 0.14 mg g^{-1} FW (flesh)	Kondo *et al*. (2005)

[a, a] – These references used 85% and 70% EtOH (v/v) extractions, respectively*

3.2.2 Extraction and quantification of sugars

Fructose, glucose and sucrose were extracted and quantified from mango peel and pulp tissue according to Terry *et al*. (2007) with slight modifications. Briefly, lyophilised samples (100 mg) were mixed well with 3 ml of 62.5:37.5 methanol (pH: 7.5): HPLC grade water (pH:7) (v/v) in 7 ml polystyrene bijou vials (Sterilin, Staffs., UK) and then placed in a shaking water bath (HAAKE SWB 20) for 15 min at 55^{o}C. Vials were removed briefly every 5 min and agitated using a vortex stirrer (Vortex Genie 2, G-560 E, Scientific Industries, NY) for 20 s to prevent layering. Samples were removed from the water bath and allowed to cool at room temperature for 10 min before subsequently being filtered using 0.2 µm Millex-GV syringe driven filter (Millipore Corp., MA). Resulting filtrates were then stored at -40ºC until needed. Extracts were diluted 1:10 with HPLC-grade water immediately before analysis.

Diluted crude mango extracts (20 µl) were injected automatically into a HPLC system comprising a P580 pump, and GINA 50 autosampler (Dionex, CA) and Rezex RCM monosaccharide Ca^{+} size exclusion column of 300 mm x 7.8 mm diameter and 8 µm particle size (Phenomenex, CA; Part no. 00H-0130-K0) fitted with a Carbo-Ca^{2+} security guard cartridge of 4 mm x 3mm diameter (Phenomenex; Part no. AJ0-4493). The mobile phase was degassed HPLC-grade water at a flow rate of 0.6 ml min^{-1}. Column temperature was held at 75°C using a Dionex STH column thermostat. Eluted

sugars from extractions were monitored by an evaporative light scattering detector (ELSD 2420, Waters, MA) connected to the Dionex system using a UCI-50 universal chromatography interface (Davis *et al*., 2007). Once the mobile phase enters the ELSD detector, it is evaporated in a heated device and the remaining solute is detected based on how it scatters light. The intensity of light scattered depends on the particle size of solid suspended particles, the solute particle size is produced based on the size of droplets generated by the nebulizer and the concentration of solute in the droplets. The presence and abundance of fructose, glucose and sucrose were automatically calculated by comparison of peak area with peak area of external standards using Chromeleon version 4.6 software (Dionex).

3.2.3 Extraction and quantification of starch

Starch concentration in lyophilised samples was measured using a total starch assay kit (Megazyme, Co. Wicklow, Republic of Ireland) according to the manufacturer's instructions (AOAC method 996.11, AACC method 76.13, ICC standard method no: 168; Saranwong *et al*., 2004). Briefly, lyophilised samples (100 mg) were mixed well with 5 ml of 80% ethanol in a centrifuge tube (plastic) and incubated at 85^{o}C for 5 min. Then, another 5 ml of 80% ethanol was added and the content was mixed well using vortex stirrer. The content was centrifuged for 10 min at 3000 rpm and the supernatant was discarded carefully. Again, 10 ml of 80% ethanol was added and stirred well before being centrifuged for 10 min at 3000 rpm. Then 3 ml of thermostable α-amylase in (Bottle 1) in MOPS buffer (50 Mm, pH 7.0) was added immediately after the supernatant was discarded and then tubes were vigorously stirred before being incubated in a boiling water bath for 6 min. The stirring was done in 2 min

intervals during incubation. The tubes were placed in a water bath at 50^{o}C and then sodium acetate buffer (4 ml, 200 Mm, pH 4.5) was added followed by amyloglucosidase (0.1 Ml, 20 U) (Bottle 2), stirred well and incubated for 30 mim. Then, entire content of the tube were transferred to a 100 ml volumetric flask and the volume was adjusted with distilled water. After mixing the content thoroughly, an aliquot of solution was centrifuged at 3000 rpm for 10 min. 1 ml of aliquot was diluted to 10 ml with distilled water and then 0.1 ml of aliquots was transferred into the bottom of the glass test tubes in duplicate. Then 3 ml of GOPOD reagent was added to each tube including the glucose control (0.1 ml glucose standard solution at 1 mg/ml) and reagent blank (0.1 ml of water), tubes were incubated at 50^{o}C for 20 min. The absorbance was measured at 510 nm for each samples using an UV/vis spectrophotometer (Camspec Ltd., Cambs., UK). Starch was analysed in only three cultivars (Willard, Karutha Colomban and Malgova) as they were identified as having the greatest differences in chemical composition.

3.2.4 Extraction and quantification of organic acids

Organic acids were extracted and quantified from mango peel and pulp tissue according to Terry *et al.* (2007) with slight modifications. Freeze-dried samples (50 mg) were mixed well with 3 ml of HPLC-grade water (pH: 7) in vials and the slurry left to stand for 5 min at room temperature. Samples were then agitated for 30 s using vortex stirrer. The slurry was filtered using 0.2 μm filter into vials and then stored at -40^{o}C until the use within 6 months.

Mixed calibration standards of organic acids such as ascorbic, citric, malic, oxalic and tartaric acid were prepared at the concentration of 0.05, 0.1, 0.5, 1.25 and 2.5

mg ml^{-1}. Mango extract (20 µl) were injected automatically into the HPLC system previously described, and Alltech Prevail Organic Acid column 250 mm x 4.6 mm diameter, 5 µm particle size (Alltech, CA; Part no. 88645) with an Alltech Prevail Organic Acid guard column of 7.5 mm x 4.6 mm diameter (Part no. 96429). Analytical grade 0.2% HPO_3 (v/v) was used as the mobile phase at a flow rate of 1 ml min^{-1}, and filtered through a filtering mechanism (Charles Austin Pump Ltd., B105 D/E) and degassed for 20 min before being used. Column oven temperature was held at 35°C. Eluted organic acids were detected using a UVD 170S/340S (Dionex, CA). Organic acids were automatically calculated by the comparison of peak area with peak area of external standards.

3.2.5 Extraction and quantification of total phenolics (TP)

Total phenolics were extracted and measured according to the Folin-Ciocalteu Method (FCM) (Singleton and Rossi, 1965) with slight modification (Terry *et al*., 2007), based on the reduction of a phosphowolframate-phosphomolibdate complex by phenolics to blue reaction products. Briefly, freeze-dried mango samples (50 mg) were dissolved in 3 ml of ethanol (80:20; v/v) (pH: 7.3) and held in a water-bath for 2 h at 70ºC, mixing every 20 min. The solution obtained was filtered as before and the clear filtrate analysed. Twenty µl of filtrate and 3.2 ml of distilled water were mixed with 200 µl of Folin-Ciocalteu's phenol reagent followed by 600 µl of sodium carbonate (1.9 M). After 2 h incubation at room temperature (20ºC) in the dark, absorbance was measured at 765 nm using a Camspec M501 UV/V spectrophotometer (Camspec Ltd., Cambs., UK). Phenol content was estimated from a standard curve of gallic acid and results expressed as mg gallic acid equivalents (GAE) g^{-1} DW.

3.2.6 Statistical analysis

Data were subjected to analysis of variance using Genstat for Windows Version 10 (VSN International Ltd., Herts., UK). Least significant difference values (LSD; P = 0.05) were calculated for mean separation. Variations among principal treatment combinations were plotted in SigmaPlot 9.0 (Systat Software, Inc., USA). Tests for correlations between mean values for concentrations were made using Spearman's Rank Correlation. Correlations are presented with the Spearman's Correlation Coefficient (r) and P value based on a two-tailed test. Both PCA and HCA (using group average linkage) were carried out on the auto-scaled data set of each cultivar separately using MATLAB version 7.3.0.267 (R2006b) in order to understand the chemometric profile of spatial and temporal variation within each cultivar. The chemometric analysis was conducted in consultation with Dr. Conrad Bessant.

3.3 Results

3.3.1 Non-structural carbohydrates (NSC)

Fructose, glucose and sucrose varied significantly among cultivars (Table 3.2; Appendix D2). Fructose was the dominant sugar (63.71 – 129.60 mg g^{-1} DW) in all cultivars and contributed to more than half of total measured sugar present, followed by glucose (18.60 – 83.62 mg g^{-1} DW) and sucrose (19.83 – 50.53 mg g^{-1} DW). Total measured sugar was highest in cv. Malgova (259.51 mg g^{-1} DW) followed by cv. Willard (205.35 mg g^{-1} DW) and cv. Ampalavi (190.13 mg g^{-1} DW). Generally, there was no significant variation in sugar concentration according to vertical sectioning (stem end, middle and distal end) (Appendix D1). Sugar concentration was significantly

lower in peel (118.69 mg g^{-1} DW) than in pulp (202.16 mg g^{-1} DW). However, there was no significant variation between inner and outer pulp. In general, total measured sugar concentration declined significantly from immature stage (199.28 mg g^{-1} DW) to fully mature stage (162.15 mg g^{-1} DW) of mango. However, sugar concentration was relatively higher in fully mature stage versus immature stage of mango cvs. Willard and Ampalavi fruit (Appendix A.3.1)**.**

Starch concentration was significantly higher in cv. Malgova (35% DW) than cvs. Karutha Colomban (29% DW) and Willard (21% DW) fruit (Appendix D2). Even though sugar concentration was relatively low in cv. Karutha Colomban as compared to cv. Willard, starch concentration was high. Starch levels varied significantly between fully mature (38.56% DW) and immature stages (19.65% DW). Mango peel (24.42% DW) had significantly lower concentration of starch than pulp tissue (30.16% DW), however there was no noticeable difference in starch concentration between peel and pulp at immature stage. It was also noticed that outer pulp (31.62% DW) contained more starch than inner pulp (28.71% DW) (Appendix D. 12(a) and (b); Table 3.2). Dry matter as a proportion of FW increased with maturity and was significantly higher in peel (0.24 – 0.51 mg g^{-1} FW) than pulp (0.17 – 0.31 mg g^{-1} FW) tissue. Mango cv. Willard had significantly lower dry matter than that of other cultivars (Appendix A.3.1.5; Table 3.2).

3.3.2 Organic acids

Citric acid contributed about 95% of total measured organic acids present in mango cultivars tested whilst malic, oxalic and tartaric acids were found in relatively lower concentrations. Ascorbic acid was expected to be in considerable concentration in

all cultivars, however it was not properly resolved since it was unstable and co-eluted with dehydroascorbic acid and derivatives thereof. Total measured acidity was significantly higher in cvs. Karutha Colomban (134.60 mg g^{-1} DW) and Vellai Colomban (115.71 mg g^{-1} DW) than that of other cultivars tested. Peel had lower acidity (13.59 – 23.52 mg g^{-1} DW) than that of pulp (96.42 – 190.10 mg g^{-1} DW). In general, total measured acidity reduced with maturity in both peel (25.91 – 16.20 mg g^{-1} DW) and pulp (177.51 – 97.50 mg g^{-1} DW), however, and in contrast, acidity increased with maturity in cvs. Malgova (117.90 – 145.81 mg g^{-1} DW) and Ampalavi (85.10 – 94.61 mg g^{-1} DW). Inner pulp had significantly higher concentration of acids (169 mg g^{-1} DW) than outer pulp (111.81 mg g^{-1} DW) (Appendix A.3.2; Table 3.3).

3.3.3 Total Phenolics

There were no significant difference ($P > 0.05$) in TP among mango cultivars, maturity stages, vertical and horizontal transactions. However, TP was relatively high in cv. Willard (15.86 mg of GAE g^{-1} DW) as compared to other cultivars (10.15 – 12.51 mg GAE g^{-1} DW). Fully mature mangoes contained slightly lower TP (9.68 mg GAE g^{-1} DW) than immature ones (12.37 mg GAE g^{-1} DW) (Appendix A.3.3). In general, TP was weakly correlated (r = 0.29) with starch and not correlated with sugars and acids. However, citric acid was negatively correlated with TP in mango peel and pulp (Appendix D. 12(a) and (b); Table 3.3).

Table 3.2. Mean concentration of non-structural carbohydrates at different maturity stages of peel and pulp of pre-climacteric Sri Lankan mango cultivars. Samples (n=3) were taken from peel and pulp of immature, mature half and fully mature fruits. Reference to LSD values is given in appendix D.2

Cultivar	Fructose (mg g^{-1}) DW (FW)		Glucose (mg g^{-1}) DW (FW)		Sucrose (mg g^{-1}) DW (FW)		Total measured sugars (mg g^{-1}) DW (FW)		Starch (%) DW (FW)		Proportion dry Weight (g $100g^{-1}$ FW)	
	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Malgova												
Immature	113.65 (32.97)	156.60 (31.75)	69.74 (20.14)	123.42 (25.15)	27.59 (8.05)	84.69 (16.94)	210.98 (61.16)	364.71 (73.84)	18.66 (5.30)	20.05 (3.99)	28.61	19.40
Mature Half	113.47 (33.01)	149.61 (34.46)	60.34 (17.75)	103.33 (24.06)	4.22 (0.94)	59.77 (13.85)	178.03 (51.70)	312.71 (72.37)	37.75 (12.38)	44.72 (9.56)	33.47	20.65
Fully Mature	91.15 (34.41)	117.80 (33.23)	43.35 (16.26)	62.60 (17.75)	7.66 (3.02)	44.19 (10.12)	142.15 (53.69)	224.59 (61.00)	37.01 (14.60)	45.47 (10.6)	38.90	22.17
Mean	106.09 (33.46)	141.34 (33.15)	57.81 (18.05)	96.45 (22.32)	13.16 (4.00)	62.88 (55.52)	177.05 (55.52)	300.67 (69.07)	31.14(10.76)	36.75 (8.04)	32.90	20.39
Willard												
Immature	60.68 (13.68)	85.99 (14.59)	40.78 (9.17)	68.59 (11.64)	26.10 (5.32)	67.56 (11.58)	127.57 (28.17)	222.13 (37.81)	17.36 (4.15)	17.73 (2.82)	23.86	15.27
Mature Half	62.33 (15.67)	90.22 (14.36)	42.76 (10.55)	76.93 (12.22)	22.09 (5.36)	68.34 (10.94)	127.18 (31.58)	235.49 (37.52)	17.46 (4.27)	23.65 (3.88)	24.04	16.31
Fully Mature	80.39 (15.64)	111.74 (22.23)	53.37 (10.25)	94.03 (18.68)	41.12 (8.01)	46.01 (8.92)	174.87 (33.90)	251.79 (49.83)	18.17 (4.02)	25.99 (4.71)	25.67	17.96
Mean	67.80 (15.00)	95.98 (17.06)	45.63 (9.99)	79.85 (14.18)	29.77 (6.23)	60.64 (10.48)	143.21 (31.22)	236.47 (41.72)	17.66 (4.15)	22.46 (3.81)	24.52	16.51
Karutha Colomban												
Immature	55.04 (31.22)	48.14 (16.47)	29.87 (16.91)	23.61 (8.08)	3.82 (2.14)	51.41 (17.32)	88.74 (50.27)	123.16 (41.87)	20.47 (11.62)	22.40 (7.63)	38.21	25.32
Mature Half	64.72 (26.47)	91.09 (23.02)	24.92 (10.19)	41.55 (10.76)	2.16 (0.86)	26.43 (6.29)	91.81 (37.53)	159.07 (40.07)	13.96 (5.98)	16.43 (4.05)	35.33	22.95
Fully Mature	48.94 (18.32)	63.07 (22.56)	17.06 (6.23)	5.52 (2.21)	9.57 (3.51)	33.46 (11.73)	75.57 (28.06)	102.06 (36.49)	38.92 (16.86)	55.00 (15.9)	43.12	27.94
Mean	56.23 (25.34)	67.43 (20.68)	23.95 (11.11)	23.56 (7.02)	5.18 (2.17)	37.10 (11.78)	85.37 (38.62)	128.10 (39.48)	24.45 (11.49)	31.28 (9.19)	38.89	25.40
Ampalavi												
Immature	83.88 (41.00)	148.86 (45.01)	45.11 (22.0)	164.06 (49.41)	0.78(0.42)	19.08(5.81)	129.77 (63.42)	332.0 (100.23)	NM		48.33	29.74
Mature Half	41.17 (14.51)	61.12 (17.12)	23.94 (8.52)	51.51 (13.57)	6.13(2.81)	16.68(5.30)	71.24 (25.85)	129.30 (35.97)			47.50	27.87
Fully Mature	105.87 (36.20)	131.54 (38.87)	28.14 (9.93)	23.25 (7.35)	16.45(5.25)	63.86(17.26)	150.45 (51.43)	218.65 (63.47)			48.83	29.55
Mean	76.97(30.6)	113.84 (33.67)	32.40 (13.48)	79.61 (23.44)	7.79(2.83)	33.21(9.46)	117.15 (46.9)	226.25 (66.56)			50.66	31.05
Vellai Colomban												
Immature	49.84 (21.01)	71.32 (20.92)	24.59 (10.43)	43.94 (13.15)	11.21 (4.80)	16.11 (4.45)	85.64 (36.19)	131.37 (38.52)	NM		41.74	27.49
Mature Half	55.67 (24.60)	78.39 (27.09)	14.74 (6.54)	10.29 (3.46)	2.64 (1.19)	15.53 (4.67)	73.05 (32.32)	104.21 (35.21)			44.16	29.38
Fully Mature	39.32 (17.21)	67.19 (25.50)	8.91 (3.98)	5.54 (2.06)	4.31 (1.79)	48.54 (18.46)	52.54 (23.00)	121.26 (46.02)			49.27	29.88
Mean	48.28 (20.91)	72.30 (24.50)	16.08 (6.98)	19.92 (6.22)	6.05 (2.59)	26.73 (9.19)	70.41 (30.50)	118.95 (39.92)			45.06	28.92

NM: *not measured*

Table 3.3. Mean concentration of organic acids and total phenolics at different stages of peel and pulp of pre climacteric Sri Lankan mango cultivars. Samples (n=3) were taken from peel and pulp of immature, mature half and fully mature fruits. Reference to the LSD values is given in appendix D.2

Cultivars	Organic acids, DW (FW)								Total measured acids (mg g^{-1}) DW(FW)		TP (mg GAE g^{-1}) DW(FW)	
	Citric acid (mg g^{-1})		Malic acid (mg g^{-1})		Oxalic acid (mg g^{-1})		Tartaric acid (mg g^{-1})					
	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Malgova												
Immature	18.7 (3.79)	105.2 (14.01)	3.65 (0.74)	7.20 (0.15)	0.41 (0.09)	0.36 (0.03)	3.7 (1.1)	5.1 (1.0)	26.46 (5.72)	117.9 (15.19)	8.80 (1.60)	11.57 (2.15)
Mature Half	11.6 (2.38)	122.7 (16.02)	1.97 (0.43)	4.47 (0.10)	0.60 (0.12)	0.43 (0.06)	2.9 (1.0)	3.9 (0.8)	17.1 (3.93)	131.5 (16.98)	8.25 (1.60)	11.86 (2.25)
Fully Mature	9.8 (2.27)	139.7 (21.49)	2.74 (0.73)	2.19 (0.33)	0.54 (0.12)	0.72 (0.12)	4.1 (1.6)	3.2 (0.2)	17.18 (4.72)	145.81 (22.2)	8.68 (2.13)	9.37 (2.24)
Mean	13.4 (2.81)	122.52 (17.17)	2.79 (0.63)	4.62 (0.19)	0.52(0.11)	0.5 (0.07)	3.6 (1.2)	4.1 (0.9)	20.25 (4.79)	131.74 (18.1)	8.58 (1.78)	10.93 (2.21)
Willard												
Immature	17.9 (4.29)	139.35 (23.4)	0.23 (0.05)	3.7 (0.53)	2.35 (0.6)	2.62 (0.42)	12.9 (2.9)	13.05 (1.9)	33.38 (7.84)	158.72 (26.2)	14.65 (3.08)	15.75 (3.2)
Mature Half	5.4 (1.33)	81.7 (13.04)	2.04 (0.5)	0.94 (0.14)	1.23 (0.3)	1.27 (0.2)	3.8 (0.9)	4.03 (0.7)	12.5 (3.03)	87.94 (14.1)	14.88 (3.42)	16.91 (3.5)
Fully Mature	12.4 (2.25)	37.8 (6.63)	1.05 (0.23)	1.76 (0.35)	1.19 (0.27)	0.83 (0.15)	6.4 (1.4)	2.2 (0.4)	21.04 (4.15)	42.6 (7.53)	16.43 (3.79)	15.71 (3.1)
Mean	11.9 (2.62)	86.28 (14.34)	1.11 (0.26)	2.13 (0.34)	1.59 (0.39)	1.57 (0.48)	7.7 (1.7)	6.43 (1.0)	23.31 (4.01)	96.42 (15.95)	15.32 (3.43)	16.12 (3.3)
Karutha Colomban												
Immature	29.0 (8.72)	271.3 (46.96)	2.01 (0.61)	3.83 (0.65)	1.5 (0.45)	1.63 (0.28)	3.1 (1.8)	3.7 (1.2)	35.61 (11.58)	280.46 (49.09)	7.93 (2.14)	7.91 (2.1)
Mature Half	10.7 (1.91)	204.0 (15.9)	0.93 (0.17)	1.52 (0.12)	2.4 (0.55)	1.86 (0.23)	4.3 (1.8)	3.5 (0.8)	18.33 (4.43)	210.9 (17.05)	15.68 (2.76)	14.11 (3.1)
Fully Mature	14.3 (3.66)	76.4 (14.38)	0.32 (0.09)	0.28 (0.05)	1.3 (0.37)	1.18 (0.25)	0.7 (0.3)	1.0 (0.3)	16.62 (4.42)	78.9 (14.98)	9.92 (2.80)	7.06 (1.8)
Mean	18.0 (4.76)	183.9 (25.75)	1.09 (0.29)	1.88 (0.27)	1.73 (0.46)	1.56 (0.25)	2.7 (1.3)	2.7 (0.8)	23.52 (6.81)	190.05 (27.04)	11.18 (2.57)	9.69 (2.3)
Vellai Colomban												
Immature	11.8 (2.99)	233.4 (36.12)	0.73 (0.18)	4.4 (0.67)	1.43 (0.35)	1.34 (0.21)	4.2 (1.8)	6.5 (1.9)	18.16 (5.32)	245.7 (38.9)	13.87 (3.64)	14.14 (3.6)
Mature Half	6.2 (1.8)	124.8 (22.7)	2.08 (0.6)	1.8 (0.34)	0.64 (0.19)	1.16 (0.22)	0.8 (0.4)	0.8 (0.2)	9.72 (2.99)	128.6 (23.46)	16.19 (4.84)	15.68 (4.5)
Fully Mature	8.1 (2.54)	122.5 (23.28)	2.16 (0.69)	1.96 (0.38)	1.42 (0.44)	0.99 (0.19)	1.2 (0.6)	0.8 (0.3)	12.88 (4.27)	126.3 (24.15)	7.21 (2.15)	7.82 (2.3)
Mean	8.7 (2.44)	160.2 (27.37)	1.66 (0.49)	2.73 (0.46)	1.16 (0.33)	1.64 (0.21)	2.1 (0.9)	2.7 (0.8)	13.59 (4.19)	166.85 (28.84)	12.42 (3.54)	12.55 (3.4)
Ampalavi												
Immature	1.3 (0.35)	76.8 (12.97)	11.37 (3.41)	3.96 (0.62)	1.47 (0.41)	1.5 (0.26)	2.3 (1.1)	2.8 (0.9)	16.44 (5.27)	85.1 (14.75)	10.28 (2.81)	15.66 (3.7)
Mature Half	16.2 (2.79)	162.2 (12.3)	2.17 (0.44)	2.2 (0.2)	1.86 (0.6)	1.1 (0.2)	6.3 (3.0)	5.9 (1.7)	26.5 (6.31)	171.4 (14.4)	8.17 (2.41)	10.55 (2.9)
Fully Mature	6.8 (1.78)	89.3 (12.58)	2.78 (0.69)	1.54 (0.22)	1.52 (0.42)	1.22 (0.23)	2.5 (1.0)	2.2 (0.6)	13.6 (3.89)	94.26 (13.63)	5.07 (1.31)	8.97 (2.0)
Mean	8.1 (1.64)	109.4 (12.62)	5.44 (1.51)	3.3 (0.35)	1.62 (0.47)	1.27 (0.23)	3.7 (1.7)	3.6 (1.1)	18.88 (5.16)	116.92 (14.26)	7.87 (2.18)	11.73 (2.9)

3.3.4 Chemometric analysis

An investigation of all variables simultaneously was necessary to fully explore the spatial and temporal variation in the chemical composition of each cultivar. Spearman's Rank Correlation analysis typically showed significant positive correlations among variables (sugars and acids). PCA bi-plots and HCA dendrograms revealed clustering of samples according to the spatial distribution and temporal variation of NSC, organic acids and TP.

PCA of cv. Willard clearly demonstrated the clustering of samples on PC1 (which captured 72.65% of the variance) and PC2 (22.93% of the variance). Peel samples were grouped away from the pulp samples along PC1, indicating that the largest contribution to biochemical variance in this study was the fruit tissue type from which the sample was taken. Although pulp samples could not be discriminated along PC1, the samples were grouped separately into immature, fully mature and half mature pulp on PC2. There was no distinguishable variation of chemical profile observed among peel samples to discriminate different maturity stages (Figure 3.1a). A HCA dendrogram also demonstrated the same clustering of samples as it is in PCA (Figure 3.1b), providing further confidence in these findings.

Data from the other tested cultivars were also separated into clusters, however the clustering was not as distinct as for cv. Willard. Generally, peel samples contained significantly lower concentration of NSC and acids and hence were clustered separately away from pulp samples. Immature pulp samples containing higher concentration of sugars and acids were separated away from fully mature pulp samples which had relatively lower concentration of sugars and acids (Figure 3.2).

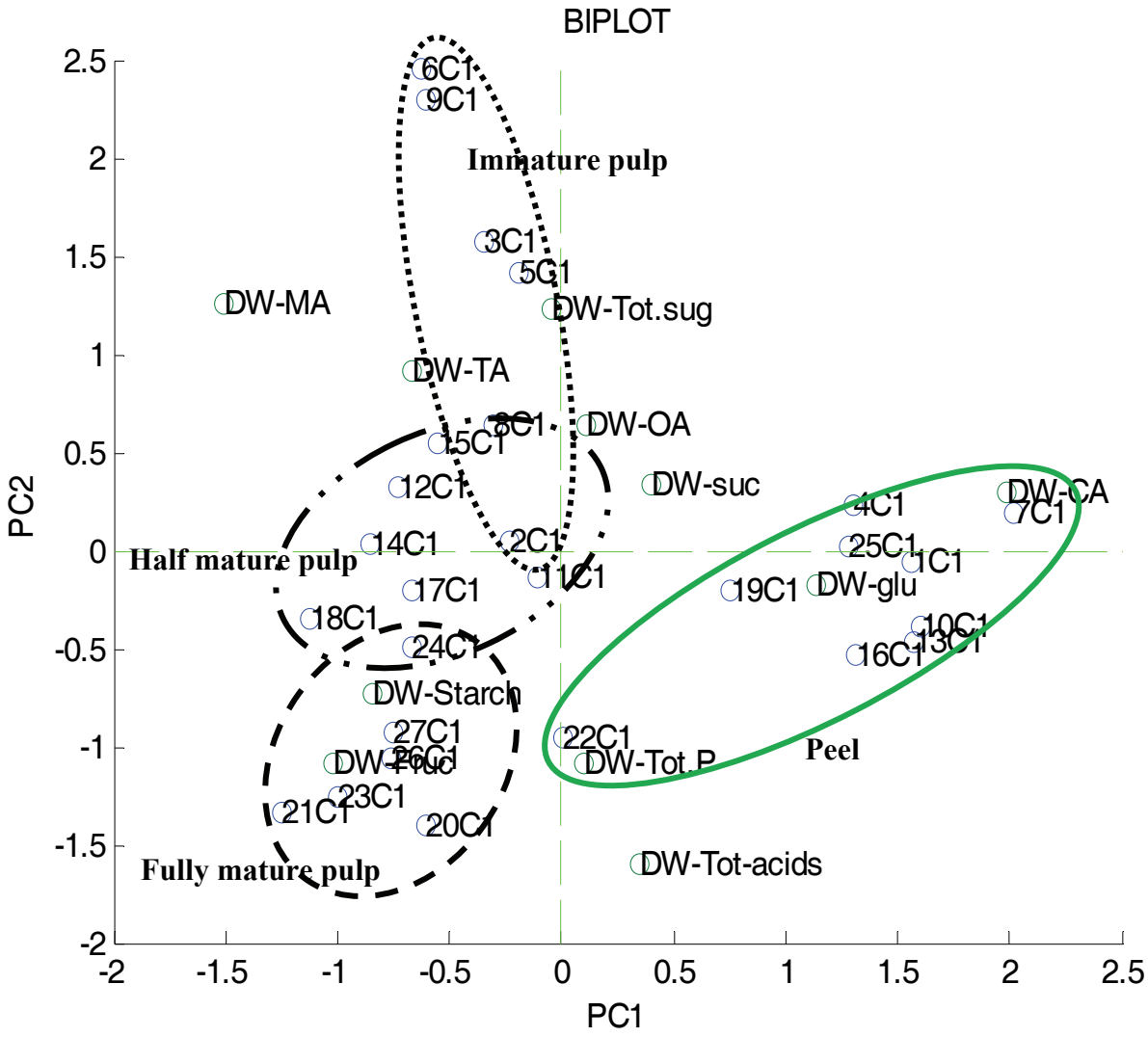


Figure 3.1(a). PCA bi-plot for PC1 (72.65%) versus PC2 (22.93%) of pre-climacteric mango cv. Willard (C1) was performed in Matlab. Samples (1C – 27C; n = 27) from peel and pulp of mango fruits at different maturity stages considered for the analysis. Grouping of samples on the loading and score plot of PCA is based on the similarities in spatial and temporal variation of sugars, acids, TP and starch. The outlines of the clusters have been added manually to aid interpretation (Appendix D3)

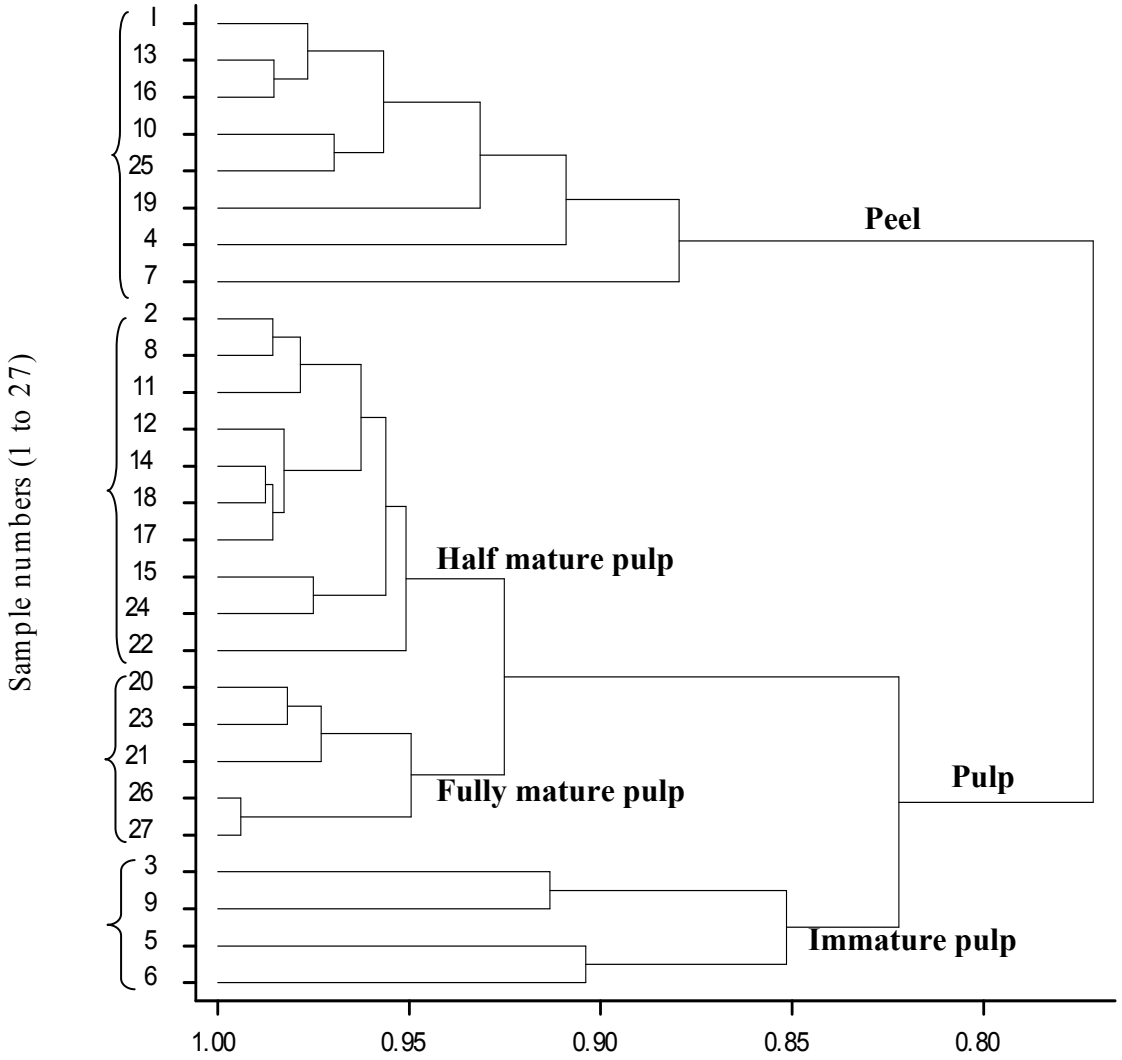


Figure 3.1(b). HCA dendrogram of pre-climacteric mango cv. Willard was performed in Genstat. Samples (1 – 27; n = 27) from peel and pulp of mango fruits at different maturity stages considered for the analysis. Dendrogram exhibits the clustering of samples based on their similarities (Appendix D3)

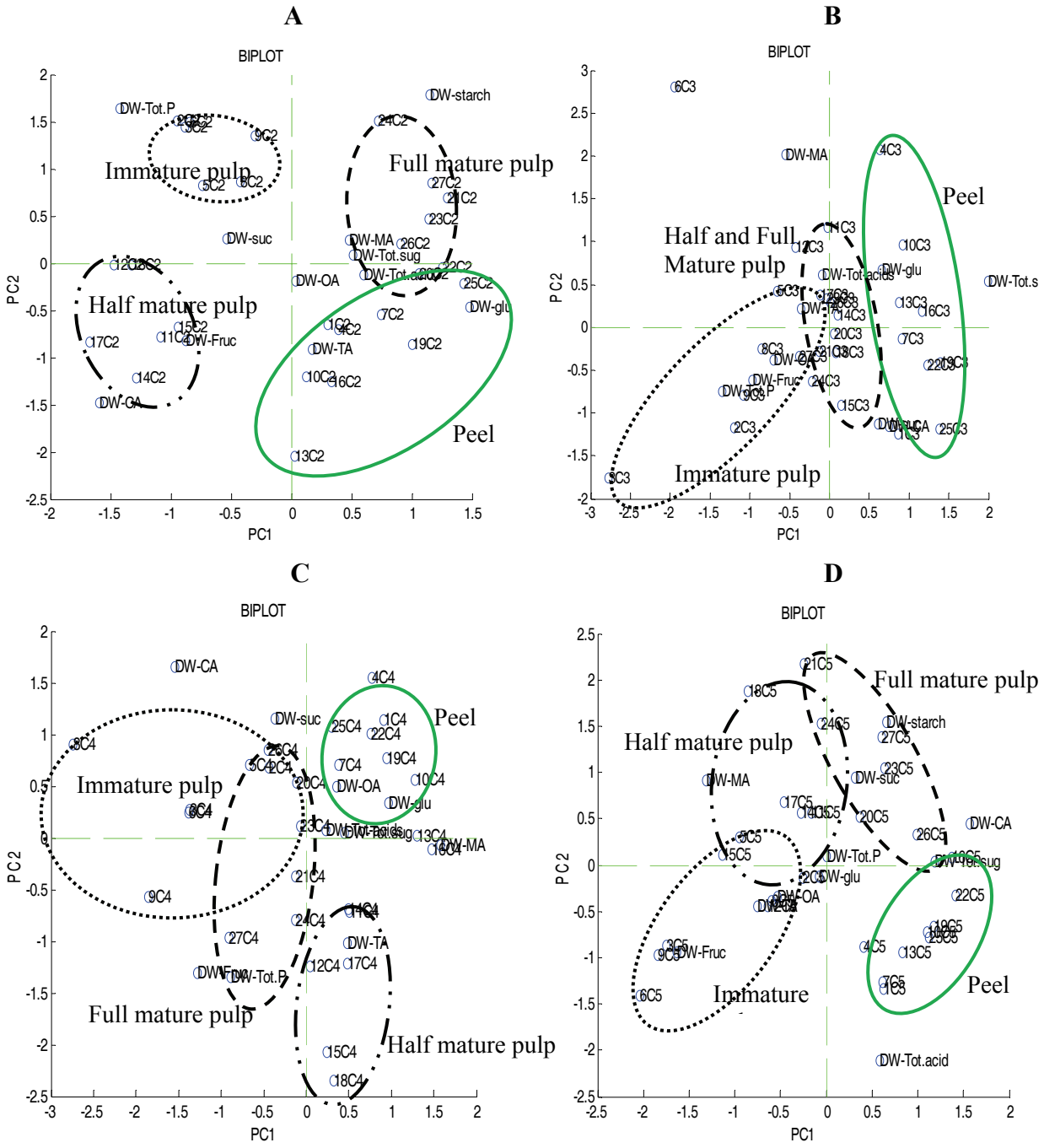


Figure 3.2. PCA bi-plots of pre-climacteric mango cvs. Karutha Colomban (A), Vellai Colomban (B), Ampalavi (C) and Malgova (D) was performed in Matlab. Clustering of 27 Samples (1C – 27C) from peel and pulp of mangoes at different maturity stages demonstrated on the loading and score plot of PCA based on the similarities in spatial and temporal variation of sugars, acids, TP and starch. The outlines of the clusters have been added manually to aid interpretation

3.4 Discussion

This is the first research that has reported that spatial and temporal variation in chemical composition of pre-climacteric mangoes can be classified using chemometric analysis. Harvest maturity determines the eating quality of fruits. Concentration of sugars and acids of ripe fruits are the prime factors which determine sweetness and consumer demand. Harvest maturity of Sri Lankan mango cultivars has been primarily determined using morphological factors (shape, colour, shoulder protrusion and appearance of the fruit) and DAFB. Since these factors vary with genotype and environment, determining the appropriate harvest maturity becomes a challenging task.

Sugar concentrations generally decreased with maturity whilst starch concentration increased. In contrast, sugar concentration increased with maturity in both peel and pulp of cv. Willard. Similarly, Saranwong *et al.* (2004) observed that total measured sugars increased from 105 to 140 DAFS in the mature green pulp samples of Thailand mango cv. Mahajanaka. Even though past studies have demonstrated that sucrose concentration is two- to six-fold higher than that of reducing sugars in the pulp samples of mature green mango cvs. Tommy Atkins, Delta R2E2, Baneshan, Swarbnarekha, Topapuri and Kensington Pride fruit (Lima *et al*., 2001; Hymavathi and Khader, 2005; Lalel *et al*., 2005; Malik and Singh, 2006), reducing sugars contributed to >80% of total measured sugar concentration in the Sri Lankan cultivars tested herein with the dominance of fructose most evident. Since the Sri Lankan mango cultivars tested in this study had higher amounts of reducing sugars than commercial cultivars, it may be expected that they perhaps achieve perceived sweetness when ripe. That said, NSCs were extracted and quantified using an aqueous methanol-based method in this study rather than using the more commonly employed ethanol-

based extraction. Reported sugar concentrations can vary significantly depending on which extraction method is used (Davis, *et al.,* 2007). Therefore, methanol-based extraction might have contributed significantly to the high final sugar concentration observed for Sri Lankan mango fruit (Appendix D 4, 5, 6 and 7).

Mango pulp samples generally contained higher concentration of NSCs than peel samples. Peel samples of cvs. Karutha Colomban, Vellai Colomban and Ampalavi had relatively higher concentrations of glucose at their fully mature stage. Though starch concentration increased with maturity in both peel and pulp, the variation between peel and pulp was relatively lower especially at the immature stage. This finding is supported by Saranwong *et al.* (2004) whereby starch concentration (using the same assay) of mango cv. Mahajanaka pulp increased by 8.86% (dry basis) from 105 DAFS to its fully matured stage at 140 DAFS. Starch is the main carbohydrate accumulated in pre-climacteric mature mango fruit, which subsequently reduces during ripening since it is hydrolysed into sugars; only trace amounts can be detected in over ripe mangoes with reduced amylase activity. Starch is digested through hydrolysis and catalysed by enzymes called amylases, which break the glycosidic bonds between glucoses in the starch polysaccharides. Mango fruit have evolved to convert starch to sugars during ripening to make fruit more attractive to seed dispersers. (Mattoo *et al*., 1975; Subramaniyam *et al*., 1976; Selvaraj *et al*., 1989; Lima *et al*., 2001). Starch concentration of cvs. Willard (23.38%), Malgova (42.65%) and Karutha Colomban (49.64%) at fully mature stage (Table 3.2) are comparable with cvs. Tommy Atkins (29.88%, Vergara-Valencia *et al*., 2007) and Mahajanaka (55.17%, Saranwong *et al*., 2004) (Tables 3.1 and 3.2). Accumulating sufficient amount of starch at mature stage would allow ripe fruit to assimilate a larger amount of sugar during postharvest

ripening. In that sense, cvs. Malgova and Karutha Colomban may result higher concentration of sugars than cv. Willard during the postharvest ripening since they have relatively higher concentration of starch. However, cv. Willard contains significantly higher content of sugars than other cultivars tested at fully matured stage. Mangoes harvested at late maturity stage are expected to have superior eating quality after ripening. It has been found that more than 75% of fruits harvested at 133 and 140 DAFS had excellent eating quality (Saranwong *et al*., 2004). Dry matter was proportional to the starch concentration and thus maturity. Both starch and dry matter should be at high concentration for the determination of harvest quality (Tandon and Kalra, 1983; Ueda *et al*., 2000).

Generally, organic acid concentration was significantly higher in the pulp than peel of the Sri Lankan mango cultivars tested. Since other acids were in relatively low concentrations in both peel and pulp, citric acid is mainly responsible for the acidic nature of mangoes. Organic acid concentration decreased during maturation, but the decreasing trend was significantly lower in cv. Malgova than other cultivars tested. Therefore, cv. Malgova contained two- to three-fold higher levels of organic acids than cvs. Willard and Karutha Colomban at fully mature stage, thus this would result in higher acidity in ripe fruit of cv. Malgova. Higher acidity at fully ripe stage would result in reduced sugar/acid ratio and ultimately would not be acceptable as a dessert fruit, and as a consequence cv. Malgova is consumed at mature stage either in fresh form or as a pickle (Table 3.3). Citric acid concentration of unripe mango cv. Alphonso pulp (24.8 mg g^{-1} FW, Yashoda *et al*., 2006) is in line with cv. Willard at the fully mature stage, but three- to five-fold lower than other cultivars tested in this study (Table 3.1).

There was no significant spatial and temporal variation observed in TP concentration (Table 3.3)**.** However, half mature mangoes had a slightly higher concentration of TP than other maturity stages, which concurs with the findings of Kondo *et al*., (2005). Since the peel has a bitterer taste than pulp, it was expected that TP would have been higher in mango peel than pulp, however the opposite was observed. Kondo *et al*., (2005) also demonstrated that peel contains higher content of TP than pulp in pre-climacteric mango fruit (Table 3.1). Combined peel and pulp tissue of mature green pre-climacteric mango cv. Tommy Atkins had similar concentration of TP (16.14 mg of GAE) (Vergara-Valencia *et al.,* 2007) with tested cultivars using a similar assay. However, TP was lower in peel and pulp of mature mango cv. Deshi, Langra, Chausa, Mallika, Deshahari and Amprapali (< 3 mg g^{-1}) (Singh *et al*., 2004). TP in the acetone extract of cvs. Raspuri and Badami peel was higher than tested cultivars (Ajila *et al*., 2007a) (Table 3.1). Since TP loosely represents dietary antioxidant capacity, pre-climacteric Sri Lankan mango cultivars may have higher antioxidant capacity than most other commercial cultivars. Higher 'antioxidant capacity' may attract more consumers as people are increasingly more concerned about the health-promoting properties of fruits. Specifically, pulp of cultivars tested had unusually higher content of TP than commercial cultivars, which is getting more attention as an edible portion of the unripe and ripe fruit. The fully mature stage of mango fruit can deemed as being the optimum harvest maturity since it contained significantly lower organic acids and higher starch content than other stages. This may increase the sugar concentration in postharvest mango fruit and enhance the sugar/acid ratio. Higher sugar/acid ratio will heighten sweetness, therefore sweeter fruit may be preferred by more consumers.

However, the spatial distribution and temporal variation of sugar concentration of mango cultivars tested was cultivar oriented.

Variation in chemical composition between peel and pulp was the major discriminatory factor amongst cultivars. The analysis differentiated pulp according to different maturity stages. In that sense, fully mature pulp samples were clustered together and had higher concentration of starch than other stages, therefore fully mature stage can be considered as the optimum stage for harvesting. PCA and HCA are 'unsupervised' approaches, meaning that no prior knowledge of the sample types is used in the analysis. The benefit of such approaches is that the clustering of samples demonstrates intrinsic variance between the samples without being biased towards desired outcomes. Chemometric analysis of foodstuffs has usually been conducted on volatile finger prints/profiles and rarely on aqueous compounds. Therefore, increased understanding of these differences and how biochemical compounds relate to one another may assist practitioners in selecting and harvesting pre-climacteric mangoes at the optimum period.

3.5 Conclusions

Fully mature mango fruit (130-140 DAFB) had the highest concentration of starch and lowest concentration of sugars and acids. Subsequently the starch is converted in to sugars during ripening. Chemometric analysis clearly revealed distinct differences according to cultivar, tissue type and maturity based upon non-structural carbohydrates, acids and total phenolics. Since these combined variables and dry matter at harvest are partially responsible for the final quality of ripe fruit, they could be used to select Sri Lankan mango fruit at the optimum stage. Selecting the appropriate harvest maturity subsequently optimises postharvest quality of ripe fruit.

CHAPTER FOUR

Temporal change in biochemical constituents of Sri Lankan mango fruits during postharvest ripening at 32^{o}C

4.1 Introduction

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in terms of production, consumer acceptance and nutritional quality. Mango has a short shelf-life and reaches a respiration peak of ripening between 3 to 4 days after harvest at ambient condition (25^{o}C) (Narayana *et al*., 1996; Rathore *et al*., 2007). However, according to Krishnamoorthy *et al*., 1971 and Yasodha *et al*., 2006, climacteric period of mango fruit extended up to a week after harvest when ripened at 28^{o}C. Yet it may take a further four days to reach a ready-to-eat ripe stage. Karutha Colomban, Willard and Ampalavi are the prominent dessert mango cultivars in Sri Lanka and are distributed throughout the country. Since long dry spells facilitates pollination, fruit set and maturity, high quality fruits are obtained in the dry zone (temperature: 30 to 34^{o}C, RH: 70 to 80% and seasonal rainfall) of Sri Lanka (Kirishnapillai, 2004).

Generally, during ripening, climacteric fruits like mango undergo significant physiological and biochemical changes such as increased respiration, ethylene production, carbohydrate depolymerisation, organic acid degradation, softening, chlorophyll degradation and biosynthesis of carotenoids and aroma compounds, etc. (Vasquez-Caicedo *et al.,* 2004; Rathore *et al.,* 2007). Though mango fruit is well known for its characteristic aroma and taste, it is also a good source of dietary antioxidants (Kauer and Kapoor, 2001; Kim *et al*., 2007) including ascorbic acid (Franke *et al*., 2004), carotenoids (Godoy and Rodriguez-Amaya, 1989) and phenolic compounds

(Berardini *et al*., 2005). Mango peel is a good source of phenolic compounds, carotenoids and other bioactive compounds which have been shown to improve human health (when extracted and consumed) by increasing antioxidant activities and reducing the incidence of cancer and heart diseases (Wolfe *et al*., 2003). However, there is no published information yet on the consumption of mango peel in the human diet. During postharvest ripening of mango, carbohydrate and acid metabolism are closely associated as acidity decreases more rapidly than sugars increase (Mizrach *et al*., 1997). Therefore, sugar/acid ratio increases during ripening. The sugar/acid ratio is also used to predict the maturity in mangoes as it is vital for the adequate taste and palatability of the fruits to be attained (Mahayothee *et al*., 2002). Fructose is the main reducing sugar in mango fruit and increases markedly during the postharvest ripening whilst sucrose (leading sugar) increases during later stage of ripening (Fuchs *et al*., 1980; Selvaraj *et al*., 1989). Citric and malic acids are the major organic acids in mango cv. Keitt and usually decrease during ripening (Medlicott and Thompson, 1985; Selvaraj *et al*., 1989). However, small concentrations of tartaric, oxalic, ascorbic and α-ketoglutaric acids have also been identified (Medlicott *et al*., 1988).

Average ambient temperature of Sri Lanka is around 30^{o}C. Therefore, postharvest life of fresh mango fruits is generally short since postharvest diseases like anthracnose and stem-end rot develop aggressively on ripening fruits at higher temperatures. It is really a challenge in Sri Lanka to extend the shelf-life of mango fruit under existing climatic conditions using rudimentary storage technology. Therefore, studies are needed to extend the shelf-life of mango fruits at higher temperature (around 30^{o}C) whilst attaining optimum quality and nutritional composition. The aim of this study was to understand the temporal change in biochemical compounds *viz.* sugars,

organic acids, TTA, TSS, sugar/acid, AsA, TP, flavonoids and total carotenoids of prominent Sri Lankan mango cultivars during postharvest ripening. Such knowledge may help growers to select the mango fruit at optimum ripening stage in terms of potential shelf-life, quality and biochemical composition.

4.2 Materials and methods

4.2.1 Plant materials sand quality parameters

Mango cultivars, endemic to Sri Lanka (Willard, Karutha Colomban and Malgova) were selected for this study based on the chemical profiles of pre-climacteric mango fruit derived from Sri Lanka (Thanaraj *et al*., 2009; Chapter 3). Mango fruits (n = 45, 15 per each cultivar) were picked at harvest maturity from the Eastern University Agronomy Farm (Batticaloa, Sri Lanka) in May 2007 and air freighted to Cranfield University, UK at ambient temperature. Upon arrival, mango fruits were subsequently ripened at 32^{o}C in a Sanyo incubator (MLR 350HT, Sanyo, Japan) for 4 days and samples were taken at each out-turn at day 0, 3 and 4 (n = 3). Both peel and pulp samples (15 to 20 g) were snap-frozen in the liquid nitrogen and stored at -40^{o}C until freeze drying. About 8 g of samples were then freeze dried using Edwards Modulyo (W. Sussex, UK) and Christ Ldc 1 freeze driers (Christ Alpha 1-4, 3360 OSTERODE, Denmark), milled to a fine powder (2 g DW) before being returned to -40°C (deep freezer) until use. Peel colour (L*, C* and h^{o}) and TSS of mango pulp were measured using a Minolta CR-400 colorimeter (Minolta Co. Ltd., Japan) and a 'Palette' digital refractometer (PR 301α, Atago Co. Ltd., Japan), respectively before ripening and at each out-turn. All the chemicals used in this analysis were purchased from Sigma (Dorset, UK) unless otherwise stated.

4.2.2 Extraction and quantification of sugars and organic acids

Fructose, glucose, sucrose and non-volatile organic acids (except AsA) were extracted and quantified from mango peel and pulp tissue according to Terry *et al.* (2007) with slight modifications (Thanaraj *et al*., 2009) as described in sections 3.2.2 and 3.2.4 of Chapter 3.

4.2.3 Extraction and quantification of total titratable acidity

Total titratable acidity was measured according to Jacobi *et al.* (2000), briefly fresh mango pulp (2.5 g, n = 33) samples were homogenised using a IKA Ultra Turrax T25 homogeniser (Janke and Kunkel, Staufen, Germany) in 5 ml of deionised water (pH: 7) and filtered using double layer muslin cloth. Filtrate (1 ml) was titrated against 0.01 M NaOH. Phenolphthalein (1% (v/v) in industrial methylated sprit; pH 10) and citric acid (1%) were added as an indicator and standard, respectively.

4.2.4 Extraction and quantification of ascorbic acid

Ascorbic acid was extracted and quantified using L-ascorbic acid assay procedure (Megazyme, Co. Wicklow, Republic of Ireland). Lyophilised freeze-dried mango samples (peel or pulp) (100 mg, n = 66) were mixed well with 5 ml of 100 mM dipotassium phosphate buffer (pH 5.6) (K_2HPO_4; prepared according to the manufacture's instructions) in 7 ml polystyrene bijou vials (Sterilin, Staffs., UK) and filtered using Surfactant-free cellulose acetate (SFCA) 0.45 μm NALGENE syringe driven filter (Nalge Nunc International, NY). Filtrate was analysed immediately using a Camspec M501 UV/vis spectrophotometer (Camspec Ltd., Cambs., UK) according to the manufacturer's instructions.

4.2.5 Extraction and quantification of total phenolics

Total phenolics were extracted and measured according to the Folin-Ciocalteu Method (FCM) (Singleton and Rossi, 1965) with slight modification (Terry *et al*., 2007; Thanaraj *et al*., 2009) based on the reduction of a phosphowolframate-phosphomolibdate complex by phenolics to blue reaction products as described in section 3.2.5 of Chapter 3.

4.2.6 Extraction and quantification of flavonoids

Flavonoids were extracted and quantified from the freeze dried peel samples (150 mg; n = 33) according to Vagen and Slimestad (2008) with slight modifications. The modification was based on optimising the extraction time. In that sense, samples were mixed well with 3 ml of 100% MeOH (pH: 7) and 15 µl of trifloroacetic acid (TFA) (pH: 2.2) using vortex stirrer and left for 4 h at ambient temperature before being extracted using 0.45 µm syringe filter. In order to optimise the extraction, samples were further extracted after 4 h at ambient temperature followed by 24 h in dark at 5^{o}C and 4 days in dark at 5^{o}C. Since the samples extracted after 24 h in dark at 5^{o}C following the 4 h at ambient temperature showed better peak, this procedure was practiced in this study. The extract was kept at -40^{o}C until analysis.

Mango extracts (10 µl) was injected automatically into the Agilent HPLC system comprising a Zorbax Eclipse XDB C18 column (150 mm x 4.6 mm diameter, 5 µm particle size) (Alltech, CA; Part no. 88645) coupled with an Zorbax Eclipse XDB guard column (17 mm x 1.0 mm diameter, 5 µm) (Alltech, CA; Part no. 96429). Analytical grade TFA (5 g l^{-1}: pH: 2.2) with HPLC water (pH: 7) (A) and TFA (5 g l^{-1}) with acetonitrile (B) (pH: 7) were used as the mobile phase at a flow rate of 0.8 ml min^{-1},

were filtered through a filtering mechanism (Charles Austin Pump Ltd., B105 D/E, UK) and degassed for 20 min before being used. The solvent gradient (85% (A) and 15% (B)) consisted of a linear increase/decrease in the amount of water in acetonitrile: 0-11 min, 15-17%; 11-20 min, 17-30%; 20-25 min, 30-80%; 25-30 min; 80-15%. Column oven temperature was held at 40^{o}C. Eluted flavonoids were detected using a diode array detector (DAD). Flavonoids were automatically calculated by comparison of peak area with peak area of external calibration standards.

4.2.7 Extraction and quantification of total carotenoids

Total carotenoids were extracted and quantified according to Malik and Singh (2006) with slight modifications. Since carotenoids are light sensitive, all steps were performed under low light. Briefly, frozen (-40^{o}C) fresh pulp samples (1 g, n = 33) were homogenised with 12% (v/v) methanolic potassium hydroxide (KOH) for 2 min in a glass test tube using an Ultra Turrax T25 homogeniser. Then samples were saponified at 37^{o}C for about 30 min. Upon removal of the sample from the water bath, 5 ml petroleum ether was added and shaken vigorously for 1 min using a vortex stirrer and left on the bench for 10 min to separate the clear supernatant. The supernatant was then transferred into a conical flask; this process was continued until the top layer of the petroleum ether became colourless. As a last step, samples were sonicated for 5 min using a SC-50-22TH sonicator (Sonicor Ins. Corp., NY). The supernatant was passed through a layer of anhydrous sodium sulphate (Na_2SO_4) placed on the sintered glass (P-160) filter in order to remove all moisture. About 3 ml of filtrate was transferred to the 10 mm HEL precision semi macro glass cuvettes (Fisher Scientific Inc., PA) and the absorbance was measured at 450 nm using a spectrophotometer.

The total carotenoids were determined using Eq. 1 (Nobre *et al*., 2006). An extinction coefficient ($E_{1cm}^{1\%}$) of pure β-carotene at 450 nm (2500 in petroleum ether) was used in the calculation.

$$x = \frac{Ay}{E_{1cm}^{1\%} \times 100} \qquad (1)$$

Where, 'x' is the amount of total carotenoids (g), 'y' is the amount of solvent (ml) and 'A' is the absorbance.

4.2.8 Statistical and chemometric analysis

Data was subjected to analysis of variance using Genstat for Window Version 10.1.0.198 as mentioned in section 3.2.6 of Chapter 3. The PCA was carried out using Unscrambler X version 10.0.1 (Oslo, Norway) on the dataset in order to understand the chemometric profile of spatial and temporal variation.

4.3 Results

4.3.1 Colour parameters, total soluble solids (TSS) and percentage weight loss

Mango cvs. Karutha Colomban (18.03%) and Willard (16%) had higher concentration of TSS than cv. Malgova (14.67%) before the start of ripening trial (Table 4.1). The peel colour parameter h^o (hue angle) decreased significantly from 86 (greenish) to 66 (yellow-orange-reddish) in cv. Willard, but h^o was significantly higher in cvs. Karutha Colomban and Malgova and reduced (98 to 75) during ripening up to day 3 and then increased. Colour parameters L* (luminosity) and C* (chroma) increased significantly in cultivars tested, however the value was significantly higher in cv.

Willard than cvs. Karutha Colomban and Malgova (Table 4.1). Percentage weight loss of whole fruit was significantly increased during ripening in cultivars tested and higher in cv. Karutha Colomban than other cultivars. Dehydration during ripening caused shrinkage on fruit peel, which was more visible on day 4 in cv. Willard.

4.3.2 Sugars and dry matter as a proportion of fresh weight

Sucrose (385.30 to 523.39 mg g^{-1} DW) was the prominent sugar and contributed 70 to 85% of total sugar concentration (545.81 to 627.70 mg g^{-1} DW) and was followed by fructose (93.49 to 124.52 mg g^{-1} DW) and glucose. Glucose concentration was lower in cvs. Karutha Colomban (10.80 mg g^{-1} DW) and Malgova (7.73 mg g^{-1} DW) than cv. Willard (66 mg g^{-1} DW). Sucrose concentration increased with ripening up to day 3 and then decreased in both peel and pulp; however, fructose significantly increased with ripening up to day 4 whilst glucose decreased (Table 4.2). Mango cv. Malgova had lower dry matter than other cultivars tested (Appendix A.4.1; Table 4.2).

4.3.3 Organic acids and total titratable acidity (TTA)

Citric acid contributed about 88.50% and 60% in the total measured organic acid concentration of pulp and peel samples, respectively. Malic, AsA, tartaric and oxalic acids were also measured in relatively lower concentrations. Peel had significantly higher concentration of malic and tartaric acid (3.24 to 6.60 and 0.86 to 5.31 mg g^{-1} DW, respectively) than in pulp (0.13 to 3.70 and 0.29 to 2.41 mg g^{-1} DW, respectively) (Appendix A.4.2). Mango cv. Willard contained about twenty-fold higher concentration of AsA *($p = 0.001$)* than cvs. Karutha Colomban (0.55 mg g^{-1} DW) and Malgova (0.40 mg g^{-1} DW) in both peel and pulp.

AsA concentration of peel (8.00 mg g^{-1} DW) was significantly higher than in pulp (5.60 mg g^{-1} DW) in mango cv. Willard. However, the variation of AsA between peel and pulp was not significant in cvs. Karutha Colomban and Malgova. AsA decreased significantly during ripening in cv. Karutha Colomban in both peel and pulp, yet increased in cv. Malgova peel (Table 4.3). Total measured organic acid concentration was significantly higher in pulp than peel (Table 4.3) and generally decreased during ripening. Citric acid decreased (85.52 to 8.80 mg g^{-1} DW) in cultivars tested whilst malic acid increased by three-fold in cvs. Karutha Colomban and Willard. Oxalic acid increased by three- to five-fold in cvs. Malgova and Willard, however no considerable variation was observed in tartaric acid concentration except in cv. Malgova (3- to 4-fold reduction). Unusually, citric acid concentration drastically decreased in cv. Malgova from day 0 (85.50 mg g^{-1} DW) to day 3 (23.7 mg g^{-1} DW) during ripening (Table 4.3). Citric acid had strong positive correlation with total measured acids (r = 0.99) and oxalic acid (r = 0.84) whilst it was negatively correlated with AsA, malic and tartaric acids. Ascorbic acid was weakly correlated with TP but strongly correlated with tartaric acid and flavonoids *viz.* quercetin 3 O galactoside and quercetin 3 O gluctoside (Appendix D. 10 and 11).

Titratable acidity was significantly higher in cvs. Malgova (10.13 mg g^{-1} FW) and Karutha Colomban (8.77 mg g^{-1} FW) than cv. Willard (5.91 mg g^{-1} FW) and decreased during ripening up to day 4 (Appendix A.4.6; Table 4.3).

Table 4.1. Variation of colour parameters (C*, L* and h^o), total soluble solids (TSS), total titratable acidity (TTA), TSS/TTA ratio and sugar/acid ratio of Sri Lankan mango cultivars during postharvest ripening at 32°C. Samples (n=3) were taken from peel and pulp before ripening (day 0), day 3 and day 4. DW – dry weight, FW- fresh weight

Cultivars	Ripening period (days)	Peel colour			TSS (%)	% weight loss (whole fruit)	TTA mg g^{-1} FW	TSS/TTA	Sugars/TTA
		L*	C*	h^o					
Willard	0	61.33	46.26	86.00	16.00	0.00	5.91	27.11	21.01
	3	62.72	60.40	67.65	18.80	9.08	2.20	85.70	65.20
	4	61.96	61.87	66.08	17.23	11.96	1.72	104.20	73.83
LSD *(P = 0.05)*		NS	3.00	8.22	2.30	0.70	0.27	15.69	16.58
Karutha	0	47.68	30.87	98.93	18.03	0.00	8.80	20.61	17.70
Colomban	3	56.36	46.01	70.99	20.53	8.94	1.74	123.94	98.33
	4	50.06	43.22	88.24	14.80	15.42	1.71	89.22	77.52
LSD *(P = 0.05)*		3.22	3.00	8.22	2.30	0.70	0.27	15.69	16.58
Malgova	0	57.30	39.03	97.69	14.67	0.00	10.13	14.53	10.91
	3	61.02	49.30	82.77	15.23	8.68	4.80	31.61	24.24
	4	59.23	49.48	87.97	11.60	10.16	2.60	44.91	51.93
LSD *(P = 0.05)*		3.22	3.00	8.22	2.30	0.70	0.27	15.69	16.58

NS: not significant

Table 4.2. Mean concentration of sugars and dry matter as a proportion of fresh weight of Sri Lankan mango (peel and pulp) cultivars during postharvest ripening at 32°C. Samples (n=3) were taken from peel and pulp before ripening (day 0), day 3 and day 4. DW – dry weight, FW- fresh weight

Cultivars	Ripening period (days)	Sucrose mg g^{-1} DW(FW)		Glucose mg g^{-1} DW(FW)		Fructose mg g^{-1} DW(FW)		Total measured sugars mg g^{-1} DW(FW)		Dry matter as a proportion of FW (g $100g^{-1}$ FW)	
		Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Kautha	0	144.4 (47.4)	523.4 (129.2)	38.8 (12.3)	10.8 (2.7)	87.7 (27.9)	93.5 (23.2)	270.9 (87.6)	627.7 (155.1)	32.4	24.7
Colomban	3	167.3 (60.7)	552.3 (140.3)	5.2 (1.9)	2.5 (0.6)	79.9 (29.2)	86.9 (22.0)	252.4 (91.7)	641.7 (162.9)	36.3	25.4
	4	109.6 (37.0)	488.0 (98.6)	2.5 (0.8)	1.9 (0.4)	88.4 (28.6)	156.0 (29.4)	200.5 (66.4)	645.9 (128.4)	31.6	19.2
Malgova	0	96.4 (26.6)	431.1 (83.8)	19.4 (5.3)	7.7 (1.7)	129.8 (35.4)	124.5 (24.5)	245.6 (67.3)	563.3 (110.0)	27.4	19.7
	3	199.0 (54.6)	524.6 (96.3)	1.0 (0.3)	1.3 (0.2)	127.7 (34. 4)	111.4 (20.4)	327.7 (89.2)	637.3 (116.9)	26.9	18.3
	4	182.3 (51.7)	544.5 (108.8)	0.2 (0.1)	0.8 (0.1)	128.0 (36.1)	125.8 (25.1)	310.5 (87.9)	671.1 (134.0)	27.7	19.8
Willard	0	140.4 (40.0)	385.3 (87.5)	40.8 (11.6)	66.0 (15.0)	61.0 (17.4)	94.5 (21.4)	242.2 (69.0)	545.8 (123.9)	28.5	22.7
	3	175.3 (60.1)	433.5 (101.3)	28.0 (9.6)	39.3 (9.1)	72.6 (24.5)	142.1 (32.5)	275.9 (94.2)	614.9 (142.9)	33.8	23.3
	4	127.1 (44.0)	356.9 (75.6)	14.7 (5.1)	42.0 (9.2)	72.2 (25.0)	174.4 (37.4)	214.4 (74.2)	573.3 (122.2)	34.7	21.3
LSD *(P = 0.05)*		58.82 (20.89)		NS(NS)		26.82 (6.60)		52.03 (23.32)			

NS: not significant

Table 4.3. Mean concentration of organic acids in peel and pulp of Sri Lankan mango cultivars during postharvest ripening at 32°C. Samples (n=3) were taken from peel and pulp before ripening (day 0), day 3 and day 4. DW – dry weight, FW- fresh weight

Cultivars	Ripening period (days)	Oxalic acid mg g^{-1} DW(FW)		Tartaric acid mg g^{-1} DW(FW)		Malic acid mg g^{-1} DW(FW)		AsA mg g^{-1} DW(FW)		Citric acid mg g^{-1} DW(FW)		Total measured acids mg g^{-1} DW(FW)	
		Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Karutha	0	2.6 (0.9)	1.2 (0.3)	0.9 (0.3)	0.7 (0.2)	4.7 (1.6)	1.6 (0.4)	1.2 (0.4)	1.1 (0.3)	12.4 (3.9)	27.0 (6.7)	21.8 (7.0)	31.5 (7.8)
Colomban	3	1.0 (0.4)	1.2 (0.3)	1.2 (0.5)	0.3 (0.1)	6.6 (2.7)	3.3 (0.8)	0.2 (0.1)	0.3 (0.1)	2.1 (0.8)	5.5 (1.4)	11.1 (4.0)	10.5 (2.7)
	4	0.9 (0.3)	1.0 (0.2)	1.4 (0.4)	0.7 (0.1)	6.4 (2.3)	1.9 (0.4)	0.1 (0.0)	0.5 (0.1)	2.1 (0.7)	5.5 (1.1)	10.8 (3.7)	9.6 (1.9)
Malgova	0	2.2 (0.6)	4.0 (0.8)	2.8 (0.8)	0.5 (0.1)	5.6 (1.5)	3.7 (0.8)	0.2 (0.1)	0.6 (0.1)	9.6 (2.6)	85.5 (16.8)	20.4 (5.6)	94.3 (18.6)
	3	1.0 (0.5)	1.1 (0.2)	1.8 (0.5)	0.5 (0.1)	6.2 (1.7)	1.3 (0.2)	0.3 (0.1)	0.2 (0.0)	2.7 (0.7)	23.7 (4.3)	12.9 (3.6)	26.8 (4.9)
	4	1.1 (0.3)	2.2 (0.4)	2.8 (0.7)	1.5 (0.3)	4.9 (1.4)	1.6 (0.3)	0.7(0.2)	0.6 (0.1)	2.8 (0.8)	8.8 (1.9)	12.3 (3.4)	14.7 (3.0)
Willard	0	1.5 (0.4)	1.3 (0.1)	5.3 (1.5)	2.7 (0.1)	3.2 (0.9)	0.7 (0.1)	8.2 (2.3)	5.8 (1.3)	6.0 (1.7)	31.1 (7.1)	24.2 (6.9)	41.6 (9.5)
	3	1.0 (0.3)	1.2 (0.3)	5.0 (1.7)	2.4 (0.5)	4.2 (1.4)	2.1 (0.5)	7.8 (2.6)	5.0 (1.1)	4.2 (1.4)	5.0 (1.2)	22.2 (7.5)	15.6 (3.6)
	4	0.8 (0.3)	1.2 (0.3)	3.4 (1.2)	1.8 (0.4)	2.9 (1.0)	1.7 (0.4)	8.2(2.8)	6.0 (1.3)	1.7 (0.6)	1.8 (0.3)	17.0 (5.9)	12.3 (2.6)
LSD *(P = 0.05)*		NS (NS)		1.00 (0.25)		NS (NS)		NS (0.22)		7.60 (2.00)		9.09 (2.54)	

NS: not significant

4.3.4 Sugar/acid ratio

Mango cv. Malgova had a relatively lower sugar/acid ratio (10.89) compared to cvs. Karutha Colomban (17.68) and Willard (20.96). The sugar/acid ratio significantly increased during ripening up to day 4 except for cv. Karutha Colomban where ratio increased up to day 3 during ripening and then decreased (Appendix A.4.8; Table 4.1).

4.3.5 Total phenolics

Total phenolics concentration of peel was about ten-fold higher than pulp in the cultivars tested. Mango cv. Willard contained significantly higher concentration of TP in both peel (83.25 mg GAE g^{-1} DW) and pulp (8.71 mg GAE g^{-1} DW). TP concentration of peel decreased significantly *($p = 0.014$)* in cvs. Willard and Malgova (62.82 to 24.01 mg GAE g^{-1} DW) during ripening, however increased up to day 3 in cv. Karutha Colomban and then decreased (Appendix A.4.3; Table 4.4). TP positively correlated with tested flavonoids except quercetin 3 O-rhamnoside ($r = - 0.63$) (Appendix D. 10).

4.3.6 Flavonoids

Mangiferin (1.62 to 7.15 mg g^{-1} DW) was the prominent flavonoid in cultivars tested followed by quercetin 3 O-glucoside (0.23 to 0.53 mg g^{-1} DW) and quercetin 3 O-galactoside (0.11 to 0.5 mg g^{-1} DW). Mango cv. Karutha Colomban contained significantly lower concentration of mangiferin, quercetin 3 O-glucoside and quercetin 3 O-galactoside than cvs. Willard and Malgova. There were no significant differences in flavonoids *viz.* quercetin 3 O-rhamnoside and kaemferol 3 O-glucoside and quercetin

among cultivars. Mango cultivars tested (except cv. Malgova) showed significant *(p = 0.002)* increase in flavonoid concentration from day 0 to day 3 during ripening and then decreased (Appendix A.4.4; Table 4.4). Flavonoids, except for quercetin 3 O-rhamnoside had a positive correlation with AsA and TP. Quercetin 3 O-galactoside highly correlated with quercetin 3 O gluctoside (r = 0.90) (Appendix D10).

Table 4.4. Concentration of total phenolics and flavonoids in Sri Lankan mango cultivars ripened at 32°C. Mangi: mangiferin, Q 3-gal: quecetin 3 O-galactoside, Q 3-glc: quecetin 3 O-glucoside, Q 3-rha: quecetin 3 O-rhamnoside, K 3-glc: Kaemferol 3 O-glucoside, Quer: quercetin

Cultivars	Ripening period (days)	Total Phenolics (mg GAE g^{-1} DW)		Flavonoids (mg g^{-1} DW)					
				Peel					
		Peel	Pulp	Mangi	Q 3-gal	Q 3-glc	Q 3-rha	K 3-glc	Quer
Willard	0	83.25	8.70	4.81	0.41	0.48	0.01	0.02	0.04
	3	84.60	8.90	5.62	0.50	0.53	0.02	0.03	0.05
	4	69.15	6.30	5.62	0.43	0.48	0.01	0.02	0.05
Karutha Colomban	0	62.20	4.20	1.62	0.13	0.27	0.02	0.02	0.03
	3	74.35	4.70	3.92	0.19	0.42	0.03	0.08	0.06
	4	60.05	3.90	1.65	0.12	0.28	0.03	0.03	0.05
Malgova	0	62.80	5.15	7.15	0.22	0.29	0.05	0.02	0.04
	3	31.85	5.15	2.35	0.17	0.27	0.04	0.01	0.03
	4	23.98	5.35	2.83	0.17	0.23	0.04	0.01	0.03
LSD (*P=0.05*)		NS		2.49	NS	NS	NS	NS	0.02

NS: not significant

4.3.7 Total carotenoids

Total carotenoid concentration was significantly higher in cv. Karutha Colomban (0.08 mg g^{-1} FW) followed by cvs. Willard (0.06 mg g^{-1} FW) and Malgova

(0.03 mg g^{-1} FW). During ripening, total carotenoid concentration increased by about four-fold in cvs. Willard and Malgova, however it was about two-fold higher in cv. Karutha Colomban (Appendix A.4.5; Figure 4.1).

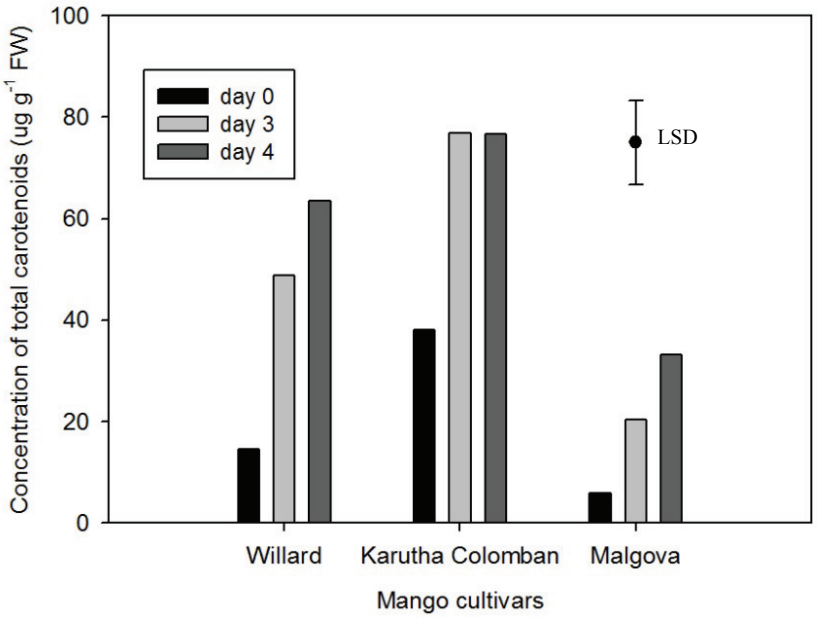


Figure 4.1. Concentration of total carotenoids in pulp samples of Sri Lankan mango cultivars during ripening at 32°C

4.3.8 Chemometric analysis

It was necessary to investigate all variables simultaneously to fully explore the genotypic, spatial and temporal variation in biochemical compounds. PCA score plot demonstrated the clustering of samples on PC1 and PC2. Based on spatial and temporal variations of taste-related compounds i.e. sugars, starch and TP, peel samples were grouped away from pulp on PC1 (captured 39% of the variance), indicating that largest contribution to biochemical variance was the samples type. However, no distinct clustering observed within cultivars except baseline pulp sample of cv. Malgova (Figure

4.2a). Peel samples showed comparatively higher genotypic variation than pulp samples, since cv. Willard peel was clustered away from other cultivars on PC2. However, no significant temporal variation was observed. According to temporal variation in health-promoting compounds of peel samples, the largest contribution to biochemical variance was cultivar, whereas cv. Willard was clustered away from the cvs. Karutha Colomban and Malgova on PC1 (59%) (Figure 4.2b). However, temporal variation was higher in cv. Karutha Colomban whilst cv. Willard showed less temporal variation.

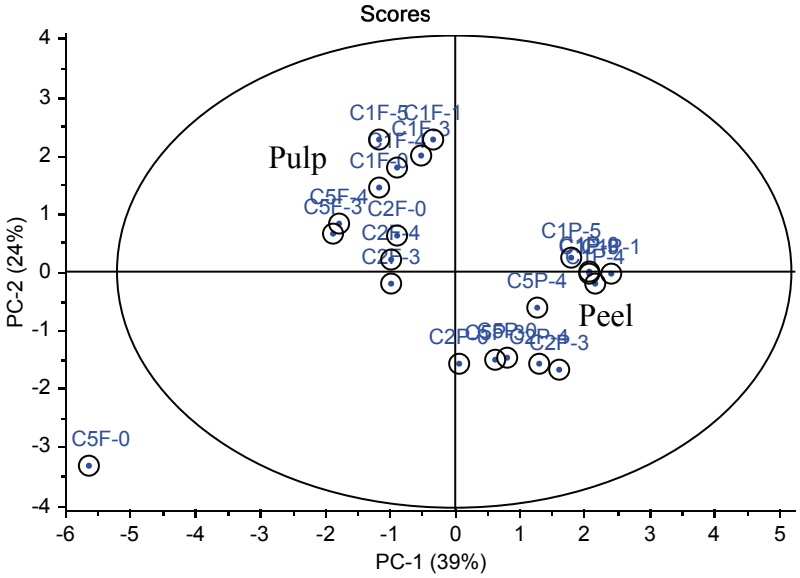


Figure 4.2(a). PCA score plot for PC1 versus PC2 of Sri Lankan mango cultivars during ripening at 32°C for 4 days was performed in Unscrambler. Grouping of samples is based on similarities in spatial and temporal variation of sugars, organic acids and TP. Hotelling ellipse shows 95% confidence. C5F-0: Malgova pulp-baseline; C5F-3: Malgova pulp-day 3; C5F-4: Malgova pulp-day 4; C5P-0: Malgova peel-baseline; C5P-3: Malgova peel-day 3; C5P-4: Malgova peel-day 4; C1F-0: Willard pulp-baseline; C1F-1: Willard pulp-day 1; C1F-3: Willard pulp-day 3; C1F-4: Willard pulp-day 4;

C1F-5: Willard pulp-day 5; C1P-0: Willard peel-baseline; C1P-1: Willard peel-day 1; C1P-3: Willard peel-day 3; C1P-4: Willard peel-day 4; C1P-5: Willard peel-day 5; C2F-0: Karutha Colomban pulp-baseline; C2F-3: Karutha Colomban pulp-day 3; C2F-4: Karutha Colomban pulp-day 4; C2P-0: Karutha Colomban peel-baseline; C2F-3: Karutha Colomban peel-day 3; C2F-4: Karutha Colomban peel-day 4

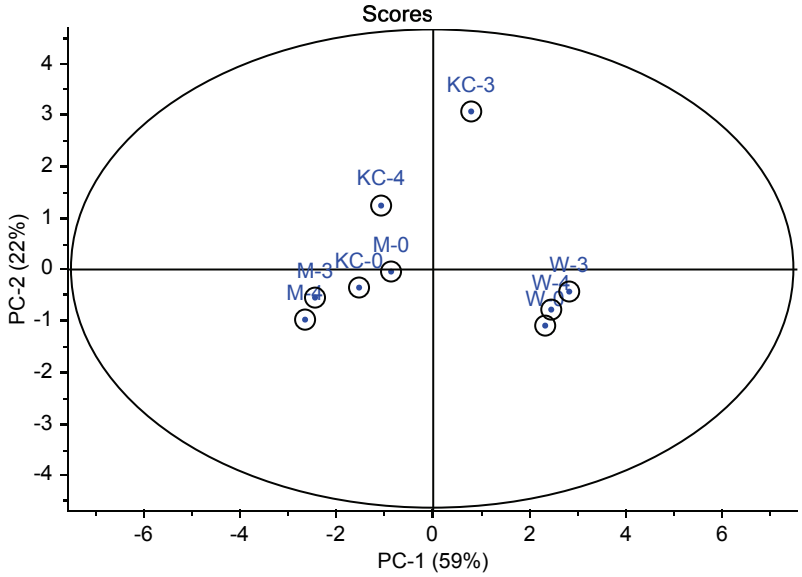


Figure 4.2(b). PCA score plot for PC1 versus PC2 of peel samples of Sri Lankan mango cultivars during ripening at 32^{o}C for 4 days was performed in Unscrambler. Grouping of samples is based on similarities in temporal variation of AsA, flavonoids and TP. Hotelling ellipse shows 95% confidence. M-0: Malgova-baseline; M-3: Malgova-day 3: M-4: Malgova-day 4; W-0: Willard-baseline; W-3: Willard-day3; W-4: Willard-day 4; KC-0: Karutha Colomban-daseline; KC-3: Karutha Colomban-day 3: KC-4: Karutha Colomban-day 4

4.4 Discussion

Mango fruit has been studied extensively, but there is a paucity of published information on the biochemical profile of Sri Lankan mango cultivars due to lack of quality research using sophisticated instruments. Therefore, more information is needed to optimise their ripening and storage potential. Total soluble solids of cvs. Willard and Karutha Colomban were significantly higher than cv. Malgova and increased in general during ripening (Table 4.1); a similar variation was also observed in mango cv. Dosehari (Rathore *et al*., 2007). Changes in TSS during ripening are associated with alteration of cell wall structure, breakdown of carbohydrates in to simple sugars and hydrolysis of starch into sugars (Kays, 1997; Kittur *et al*., 2001). Mango cv. Alphonso also showed a similar variation of TSS during ripening at 18 to 34^{o}C (Manzano *et al*., 1997; Doreyappy-Gowda and Huddar, 2001). Therefore, an increase in TSS might be an indication of the ripening process, but a decrease in TSS during the latter part of the ripening period may be due to over ripeness of fruit, when starch reserves are depleted in the over ripe mango fruits. Therefore, the accumulation of sugars may not be enough to replenish the sugars used during respiration (Medlicott and Thompson, 1985). The increase in percentage weight loss during ripening may be associated with the biochemical changes, transpiration and dehydration, which is also supported by Carrillo-Lopez *et al*. (2000) who reported that percentage weight loss increased up to 10% in cv. Haden during ripening at 13^{o}C for 32 days. Both transpiration and dehydration were also further enhanced by the higher ripening temperature, which in-turn caused shrinkage in the peel at the end of ripening period and subsequently reduced the quality. Since cv. Willard has comparatively thin peel, the impact of high temperature during ripening was more pronounced than for other cultivars tested.

Ripening temperature plays an important role in extending the shelf-life of mango fruit, however moderate ripening temperatures i.e 25^{o}C and 28^{o}C showed considerable variation in mango shelf-life (Krishnamoorthy *et al*., 1971; Narayana *et al*., 1996; Yasodha *et al*., 2006; Rathore *et al*., 2007). This difference may be due to the influence of RH, ethylene, light, etc during ripening process.

The change in mango fruit colour is an indicator of fruit maturation and ripening stage (Ueda *et al*., 2000) and is considered as one of the important parameters that determine fruit quality. Higher values for L* and C* and a lower value for h^{o} of cv. Willard indicate better colour development than cvs. Karutha Colomban and Malgova. A significant increase of C* and L* whilst a decrease of h^{o} during ripening demonstrate an improvement of colour properties in the cultivars tested (Table 4.1). A decrease in h^{o} value revealed changes in mango fruit colour from greenish to yellow and/or orange/reddish. The colour parameter h^{o} of mango cv. Kent (104.6) was comparable with cvs. Karutha Colomban and Malgova (Tovar *et al*., 2001). The L* value increased whilst C* value dropped during ripening at 23^{o}C in cv. Kent, but both C* and L* values increase in cultivars tested and thus increase the colour purity. The changes in mango peel colour are probably due to the physico-chemical changes such as degradation of chlorophyll and the synthesis of pigments *viz*. carotenoids, anthocyanins, xanthophylls and lycopene. The disappearance of chlorophyll and the accumulation of total carotenoids and other pigments improve the colour and attractiveness of mango fruit. Since the peel colour changes in to yellowish orange or red (more prominent in cv. Willard than other cultivars tested), it may attract more people. Mango is a rich source of carotenoids, which are responsible for the yellow to orange colour in ripe fruit and substantially contributes β-carotene in the diet mainly in tropical countries as it is one of

the main dessert fruits (Godoy and Rodriguez-Amaya, 1994; Marin *et al*., 1992; Cano and de Ancos, 1994). β-carotene is the most common form of carotenoid that can be found in yellow, orange, and green leafy fruits and vegetables. Carotene is an antioxidant and also protects plant cells against destructive effects of ultraviolet light. Average daily intake of β-carotene is in the range of 2-7 mg. However, the intake of β-carotene at about 30 mg day^{-1} (10 times of daily intake) has been shown to increase the risk of lung cancer and prostate cancer in smokers and people with a history of asbestos exposure (Koushik *et al*., 2006).

Total carotenoid content of mango cultivars tested increased significantly during ripening whilst the concentration in cv. Karutha Colomban and Willard was in line with the cvs. Sindhri (0.07 mg g^{-1} FW) and Chaunsa (0.06 mg g^{-1} FW), respectively (Maqbool and Malik, 2008). It was supported by Mercadante and Rodriquez-Amaya (1998) that composition and concentration of carotenoids vary with cultivars, climactic effects, stage of maturity, fruit processing and storage conditions. Litz (2009) reported that total carotenoid content usually varies from 0.01 to 0.09 mg g^{-1} FW in most mango cultivars, however Indian mango cv. Alphonso shows exceptionally higher concentration (0.11 mg g^{-1} FW) (Padmini and Prabha, 1997). Therefore, cvs. Willard and Karutha Colomban fruit pulp attain more attractive colours (orange-yellow) with good appearance during the postharvest ripening. In addition, the increase in colour properties may enable fruit to be dispersed by the birds and rodents. Therefore, cvs. Willard and Karutha Colomban may attain more benefits from carotenoids since they contain relatively higher total catotenoids.

During ripening of mango fruit, sugars increase whilst starch and acidity decrease (Medlicott and Thompson, 1985; Yashoda *et al*., 2006). Sugars and acids of

ripe fruits are the prime compounds which are responsible for the sweetness of mango fruit. Sucrose was the leading sugar in cultivars tested and increased during ripening, but glucose content reduced. Sucrose is synthesised due to the breakdown of starch during ripening (Morga *et al.*, 1979), however it is believed that sucrose may also be synthesised during ripening from hexose phosphates via sucrose-phosphate synthesis (Castrillo *et al*., 1992). The marked decrease in glucose during ripening may be associated with respiration (not measured). Sugars and organic acids are the main substrates for the respiration process in plants (Tucker and Grierson, 1987). Variation of sugars and acid concentrations during ripening of Sri Lankan mango fruits was in agreement with Castrillo *et al*. (1992) and Medlicott and Thompson (1985), respectively. That said, the concentration of sugars in mango cv. Haden (110 mg g^{-1} FW) was in line with the cultivars tested in this study. Glucose concentration is generally lower in most of the mango cultivars and contributed to relatively a smaller fraction in total measured sugars except cvs. Alphonso (Yashoda *et al*., 2006) and Willard (Table 4.2). According to the Medlicott and Thompson (1985), citric and malic acids are the main organic acids in mango cv. Keitt, however tartaric, oxalic and ascorbic acids are also present in lower concentrations. The organic acid content was also in agreement with Shashirekha and Patwardhan (1976), who found that citric acid was the major organic acid in mango cv. Badami and decreased by about ten-fold during ripening along with a slight increase in malic acid. Citric acid concentration of mango cv. Ataulfo (29.12 mg g^{-1} DW; Montalvo *et al*., 2007) was in line with cvs. Karutha Colomban and Willard whilst three-fold lower than cv. Malgova. However, mango cv. Alphonso had three- and eight-fold lower concentration of citric acid than cvs. Karutha Colomban and Malgova, respectively (Yashoda *et al*., 2006).

The concentrations of total measured sugars in the cultivars tested was in line with mango cv. Alphonso (Yashoda *et al*., 2006) and cv. Chiin Hwang No1 (Ueda *et al*., 2001). However, total measured sugars were about two-fold higher than measured in cvs. Baneshan, Suwarnarekha and Totapuri with 86% contribution of sucrose (Hymavathi and Khader, 2005) and four-fold higher than that of cv. Kensington Pride with 73% contribution of sucrose (Malik and Singh, 2006). The partial breakdown of pectin, cellulose (Roe and Bruemmer, 1981) and other polysaccharides (Kalra and Tandon, 1983) may also contribute to the rise of sugar levels during ripening. Variation of TTA during ripening was in agreement with the findings of Doreyappa-Gowda and Huddar (2001) and Srinivasa *et al*. (2002). The TTA is generally higher in Sri Lankan cultivars tested herein than commercial cultivars; however mango cv. Dosehari (Rathore *et al*., 2007) which were also ripened at 32^{o}C to 35^{o}C for 15 days had a similar concentration. Decrease in acidity during ripening might be due to citric acid degradation and further utilization in metabolic process in fruits (Rathore *et al*., 2007).

The AsA content of Mango cvs. Karutha Colomban and Malgova was comparable with some Brazilian, Indian and commercial mango cultivars (Thomas and Oke, 2007), however cv. Willard contained significantly higher levels than other cultivars. The variation of AsA content during ripening was mostly based on genotype and was in agreement with the findings of Gomez and Lajolo (2008) on mango cv. Keitt, Diallinas *et al.* (1997) and Carrillo-Lopez *et al*. (2000) on cvs. Haden, Palmer and Van Dyke. Therefore, cv. Willard may be perceived to be preferred for export than the other cultivars tested since it contains higher sugars and AsA. Ascorbic acid usually acts as an antioxidant as it is available for energetically favourable oxidation. Oxidant like hydroxyl radical contains a highly reactive unpaired electron. This can damage humans

and plants at the molecular level due to the possible interaction with nucleic acids, proteins and lipids. Ascorbic acid terminates radical reactions by serving as a stable (electron + proton) donor in interactions with free radicals, being converted into the radical ion called "semidehydroascorbate" and then dehydroascorbate. In addition to direct antioxidant effects, AsA is also a substrate for the redox enzyme ascorbate peroxidase, a function that is particularly important in stress resistance in plants (Valpuesta and Botella, 2004). Gomez and Lajolo (2008) have also reported that AsA content increases from early stage of the fruit development to harvest maturity. However, in general, there is no significant variation in AsA content after the harvest, but it decreases slightly at the final stage of ripening (Gomez and Lajolo, 2008). This profile is closely related with L-galactono-1, 4-lactone dehydrogenase (GalLDH) activity, which catalyses the last step of AsA synthesis. GalLDH activity decreases during ripening between two to four days after the harvest and then return back to initial level. However, GalLDH was not measured in this study. Hancock and Viola (2005) and Wang *et al*. (2009) have reported that AsA content of mango decreases by half during ripening.

Sweetness (sugars) and sourness (organic acids) are responsible for the taste of many fruits (Kays, 1997). The sugar/acid ratio was relatively low in cv. Malgova due to the higher concentration of acids than cultivars tested and in general the sugar/acid ratio increased due to the marked decrease of acids (Table 4.1). Mizrach *et al*. (1997) supported that carbohydrate and acid metabolism are closely associated during postharvest ripening as acidity decreases more rapidly than sugars increase. Krishnapillai (2004) reported that Sri Lankan mango cvs. Karutha Colomban (131.91) and Willard (165.30) with relatively higher TSS/TTA ratio (131.91 to 165.30) have

excellent taste scores during ripening at 32^{o}C. However, these values were noted as relatively higher than the values obtained from the present study for cvs. Karutha Colomban (123.91) and Willard (104.20). The variation in taste score might be due to the fluctuations in acids, pH, sugars and sugar/acid ratio. Sugars and acids are the primary taste compounds (Malundo *et al*., 2001). That said the cvs. Willard and Karutha Colomban are assumed to be sweeter than cv. Malgova, but a taste panel would have to be conducted to verify this. Generally, TP of mango fruit decreases during ripening (Kim *et al*., 2007). In contrast, during low temperature storage, total phenolics decrease but have no significant correlation with antioxidant capacity (Shivashankara *et al*., 2004). Total phenolic concentration of cvs. Karutha Colomban and Malgova peel was in line with Brazilian mango cv. Uba (57.24 mg GAE g^{-1} DW; Ribeiro *et al*., 2008), however it was about one- to two-fold higher than cvs. Raspuri (46.31 mg GAE g^{-1} DW) and Badami (33.30 mg GAE g^{-1} DW) (Ajila *et al*., 2007a). Peel of cv. Willard contained relatively higher concentration of TP than most of other mango cultivars, but it was comparable with cv. Hayden (70 mg GAE g^{-1} DW) (Ajila *et al*., 2007b). Ueda *et al*. (2001) reported that TP content was higher in peel than pulp at any stage of fruit development, however there were no significant difference between peel and pulp in Sri Lankan mango cultivars (Karutha Coloman, Willard, Malgova, Vellai Colomban and Ampalavi) up to the harvest maturity (Thanaraj *et al*., 2009). Although mangiferin concentration of cultivars tested was several folds higher than cv. Uba (0.20 mg g^{-1} DW), Q 3 O-glucoside, Q 3 O-galactoside, Q 3 O-rhamnoside, kaemferol 3 O-glucoside and quercetin content of cvs. Karuthe Colomban and Malgova were comparable with cv. Uba (Ribeiro *et al*., 2008). However, cv. Willard contained about two- to three-fold higher concentration of Q 3 O-glucoside and Q 3 O-galactoside than cv. Uba (Ribeiro *et*

al., 2008). Mangiferin content of cv. Karutha Colomban was in line with the peel samples of cv. Tommy Atkins (1.69 mg g^{-1} DW), but two- to three-fold lower than cvs. Willard and Malgova. Quercetin 3 O-glucoside and Q 3 O-galactoside content of cvs. Karutha Colomban and Malgova were about two- to three-fold lower than cv. Tommy Atkins whilst cv. Willard was comparable with cv. Tommy Atkins in terms of these compounds (Berardini *et al*., 2005). Specifically cv. Willard peel has relatively high TP and flavonoid content compared to other cultivars, therefore, Sri Lankan mango fruit peel (often regarded as waste during processing) could be regarded as being a potential secondary source of antioxidants. Quercetin is more specifically a flavonol, and is the aglycone form of other flavonoid glycosides, such as rutin and quercitrin. Although there is a preliminary clinical evidence that asthma, lung cancer and breast cancer are lower among people consuming relatively higher dietary levels of quercetin, detailed studies show that there is no physiological role (no appropriate physical evidence for lowering of said diseases). The finding of this study was in agreement with Haard and Chism (1996) and Kim *et al*. (2007) as total phenolics and flavonoid concentration of tested cultivars reduced consistently during ripening.

Pulp samples were clustered away from peel on PC1. Indistinct clustering between cultivars indicated that genotype and ripening period had less variance than spatial variation except for peel samples of cv. Willard since they contained higher AsA and TP than other peels. However, baseline pulp sample of cv. Malgova was clustered away from all other samples as it contained significantly higher content of organic acids than all other cultivars. Therefore, the PCA (Figure 4.2a) demonstrated spatial variation in biochemical compounds rather than genotypic and temporal variations. Distinct clustering of cv. Willard peel from other peel samples indicated that genotype (higher

AsA, TP, flavonoids and total carotenoids) had higher variance than temporal variation. However, ripening period was of more influence on the variation of health-promoting properties in cv. Karutha Colomban peel than other cultivars. Therefore, PCA (Figure 4.2b) revealed genotypic variation over temporal variation. Increased understanding of these differences and knowledge about how taste- and health-related compounds relate to one another may assist growers and indeed breeders in selecting and developing new cultivars.

4.5 Conclusions

The sugar/acid ratio increased during ripening as the decrease of acids was comparatively higher than the increase in sugars. Mango cv. Willard fruit had higher sugar/acid ratio and better peel colour than cvs. Karutha Colomban and Malgova. Therefore, cv. Willard may be perceived as a superior cultivar by exporters and consequently consumers since a higher sugar/acid ratio improves the sweetness. The TP and flavonoids decreased during ripening whilst total carotenoid content increased. Mango cv. Malgova contained comparatively lower total carotenoids than other cultivars tested whilst cv. Willard had exceptionally higher AsA content. In general, mango cvs. Willard and Karutha Colomban contained significantly higher concentration of AsA, Total carotenoids, TP and flavonoids than cv. Malgova. Therefore, cvs. Willard and Karutha Colomban could be selected for export since they have higher concentrations of biochemical constituents that have been linked to taste preference than most commercially available mango cultivars. Though higher temperature ripening commonly enhances quality parameters and biochemical composition of mango fruits, it can not be recommended since the final quality of mango fruits were detrimentally affected during the latter part of the ripening period.

CHAPTER FIVE

Temporal change in biochemical profile of Sri Lankan mango fruit (*Mangifera indica* L.) during postharvest ripening at different temperature

5.1 Introduction

Generally, climacteric fruits like mango undergo biochemical changes (ethylene production, carbohydrate depolymerisation, organic acid degradation, softening, chlorophyll degradation and biosynthesis of carotenoids and aroma compounds) during ripening (Herianus *et al.,* 2003; Vasquez-Caicedo *et al.,* 2004; Rathore *et al.,* 2007). The climacteric period of mango fruit extends up to 8 days after harvest and ready-to-eat ripe stage is achieved by day 12 of ripening at 28^{o}C (Krishnamoorthy *et al*., 1971; Yasodha *et al*., 2006). The shelf-life of mango fruit varies depending on storage conditions and cultivar, and ranges from 4 to 8 days at ambient temperature (30^{o}C) and 2 to 3 weeks in cold storage at 13^{o}C (Carrillo-Lopez *et al*., 2000).

Willard and Karutha Colomban are the prominent dessert mango cultivars endemic to Sri Lanka. High quality fruits are obtained in the dry zone (temperature: 30–34^{o}C, RH: 70-80% and seasonal rainfall) of Sri Lanka, since long dry spells facilitates pollination, fruit set and maturity (Kirishnapillai, 2004). Mango cv. Willard has higher TSS, pH and total titratable acidity (TTA) than other popular Sri Lankan cultivars (Kirishnapillai, 2004). Sugars constitute 91% of the soluble solids from mesocarp of the ripe mango cv. Ngowe (Brinson *et al.,* 1988). During postharvest ripening of mango, carbohydrate and acid metabolism are closely connected and acidity decreases more rapidly than sugars increase (Mizrach *et al*., 1997). Citric and malic acids are the major

organic acids in mango cv. Keitt and usually decrease during ripening (Medlicott and Thompson, 1985; Selvaraj *et al*., 1989). However, considerable concentrations of tartaric, oxalic, ascorbic and α-ketoglutaric acids have also been identified (Medlicott *et al*., 1988).

Mango fruit is generally a good source of dietary antioxidants (Kauer and Kapoor, 2001; Kim *et al*., 2007). However, mango peel is rich in phenolic compounds, carotenoids and other bioactive compounds which may have implications towards human health if used as secondary products (Wolfe *et al*., 2003). Fruit antioxidants play an important role in reducing the risk of degenerative diseases such as arthritis, cancer, alzemers disease, osteoporosis, heart diseases, diabetes, etc. (Shivashankara *et al.,* 2004). Antioxidant capacity of fruits varies with genetic differences, stage of harvest, season of harvest and postharvest storage condition and processing (Shivashankara *et al*., 2004).

The aim of this study was to understand the spatial and temporal changes of biochemical compounds of Sri Lankan mango fruits during ripening at 30^{o}C and 20^{o}C. Mango fruits are commonly ripened in Sri Lanka under ambient conditions (± 30^{o}C). However, mangoes are widely sold on the global market at lower temperatures (± 20^{o}C). Mango fruits are displayed at retail around 20^{o}C in most commercial food stores. In addition, there may be a possibility for temperature shock before and after storage. Therefore, it was necessary to study the possible variation in quality and nutritional compounds of mango fruits during ripening at 30^{o}C and 20^{o}C. This study may help to determine the optimum ripening conditions for mango fruit as applicable for tropical countries which often do not have refrigerated storage facilities.

5.2 Materials and methods

5.2.1 Plant materials

Mango cvs. Willard (n = 21) and Karutha Colomban (n = 15) fruits were picked at harvest maturity from the Eastern region (Batticaloa) of Sri Lanka and air freighted to the UK in uncontrolled atmospheric package in June 2008. Mangoes reached the Plant Science Laboratory, Cranfield University within four days from the harvest date. However, most of the mangoes were at fully mature stage upon arrival. The baseline temperature of mango fruits was 23^{o}C on arrival, but the relative humidity was not measured. The temperature during transit was not monitored. Mango fruits were subsequently ripened at either 20^{o}C or 30^{o}C in temperature control rooms for 6 days. Ripening temperature was changed (swapped) at day 3 of ripening from 20^{o}C to 30^{o}C and 30^{o}C to 20^{o}C (this stage of temperature change was not performed for cv. Karutha Colomban due the shortage of fruits) in order to understand the impact of temperature shock during ripening (Table 5.1). Since mango is a climacteric fruit, the ethylene production and respiration rate were expected to increase during ripening, but was not measured in this study as it was impossible to measure the ethylene production and respiration rate of fruit prior to transit due to facilities are not being available and indeed during the transport of fruits from Sri Lanka. Firmness of mango fruit was also expected to change during ripening, however it was not measured as most of methods are destructive and many mango fruits would have been needed. The research was limited to biochemical analysis alone due to budgetary constraints. Furthermore, mango fruits reached Cranfield after 4 days from harvesting date and the firmness was not measured

during that period due to time pressures. Change in firmness during ripening is an important parameter that influences final quality of fruit.

The relative humidity was recorded as between 60 to 70% in the temperature control rooms during ripening. Fruits were ripened in the dark. Out-turns were made at days 0, 3 and 6 during ripening (n = 3). Whole mango fruits were weighed using a precise electronic balance (3000C-6000D, Switzerland) before ripening and at each out-turn. The objective colour of mango peel (L*, C* and h^o) and TSS of mango pulp were measured using a Minolta CR-400 colourimeter (Minolta Co. Ltd., Japan) and a 'Palette' digital refractometer (PR 301α, ATAGO Co. Ltd., Japan), respectively before ripening and at each out-turn. Peel (15 g FW) and pulp (20 g FW) samples were snap-frozen in liquid nitrogen and stored at -40^{o}C until freeze drying. Samples were then freeze-dried using the Edwards Modulyo (W. Sussex, UK) and Christ Ldc 1 freeze driers (Christ Alpha 1-4, 3360 OSTERODE, Denmark), milled to a fine powder (2 g DW) before being returned to -40°C until use. All the chemicals used in this analysis were purchased from Sigma (Dorset, UK) unless otherwise stated.

5.2.2 Extraction and quantification of sugars

Fructose, glucose and sucrose were extracted using 62.5% (v/v) aqueous methanol and quantified from freeze-dried mango samples (100 mg, n = 72 from peel and pulp) according to Terry *et al.* (2007) with slight modifications (Thanaraj *et al*., 2009). Diluted crude mango extracts (20 µl) were injected automatically into an Agilent 1200 series HPLC binary pump system equipped with an Agilent refractive index detector (RID) and a Rezex RCM monosaccharide Ca^+ size exclusion column of 300 mm x 7.8 mm diameter and 8 µm particle size (Phenomenex, CA; Part no. 00H-0130-

K0) fitted with a Carbo-Ca^{2+} security guard cartridge of 4 mm x 3 mm diameter (Phenomenex; Part no. AJ0-4493). Degassed HPLC-grade water was used as mobile phase at a flow rate of 0.6 ml min^{-1} (Terry *et al.,* 2007; Thanaraj *et al*., 2009). Column temperature was held at 80°C using a G1316A thermostatic column compartment. The presence and abundance of sugars were automatically calculated by comparison of peak area with peak area of external standards (0.05 to 2.50 mg ml^{-1}) using ChemStation rev. B.02.01.

5.2.3 Extraction and quantification of organic acids

Non-volatile organic acids (oxalic, tartaric, malic, AsA and citric) were extracted using HPLC-grade water (pH 7) from freeze-dried mango samples (50 mg, n = 72 from peel and pulp) according to Terry *et al.* (2007) with slight modifications (Thanaraj *et al*., 2009). Diluted mango extracts (20 µl) were injected automatically into Agilent 1200 series HPLC binary pump system equipped with a diode array detector (DAD) and an Alltech Prevail Organic Acid column 250 mm x 4.60 mm diameter, 5 µm particle size (Alltech, CA; Part no. 88645) with an Alltech Prevail Organic Acid guard column of 7.50 mm x 4.60 mm diameter (Alltech, CA; Part no. 96429). Analytical grade 0.2% HPO_3 (v/v) was used as the mobile phase at a flow rate of 0.60 ml min^{-1}, was filtered through a filtering mechanism (Charles Austin Pump Ltd, B105 D/E, England) and degassed for 20 min before being used. The presence and abundance of organic acids were automatically calculated by comparison of peak area with peak area of external standards (0.04 to 1 mg ml^{-1}) using ChemStation rev. B.02.01.

5.2.4 Extraction and quantification of total phenolics, flavonoids and total carotenoids

Total phenolics were extracted and measured as described in section 3.2.5 of chapter 3 whilst flavonoids and total carotenoids were extracted and analysed as described in sections 4.3.7 and 4.3.8 of Chapter 4, respectively.

5.2.5 Statistical and chemometric analysis

Data was subjected to analysis of variance using Genstat for Window Version 10.1.0.198 (VSN International Ltd., Herts., UK) as mentioned in section 3.2.6 of chapter 3. The PCA was carried out using Unscrambler X version 10.0.1 (Oslo, Norway) as described in section 4.2.7 of Chapter 4.

Table 5.1. Treatments and sampling structure of mango fruits ripened at 20°C and 30°C

Cultivars	Mango fruits (Nos)	Baseline sampling (day 0)	Ripening Temperature (°C)	Ripening period (days) 1 2 3 4 5 6
Willard	21	S1	20	9 S2 S3 S3
			30	9 S3 S3 S2
Karutha Colomban	15	S1	20	9 S2 S3
			30	3 S3 S3

-Ripening at 20°C; - Ripening 20°C and swapped to 30°C at day 3; - Ripening at 30°C; - Ripening at 30°C and swapped to 20°C at day 3. S1: Sampling (3 fruits in each cultivar) before ripening trial; S2: Sampling (n=3) at day 3 during temperature swap; S3: Sampling (n=3) at the end of each temperature treatment

5.3 Results

5.3.1 Colour and TSS

Mango cv. Willard (14.95%) contained higher TSS than cv. Karutha Colomban (13.50%). The TSS increased during ripening in cv. Willard, but did not change in cv. Karutha Colomban. However, generally there was no significant difference in TSS values during ripening at different temperatures (Table 1). The peel colour parameter h^o (hue angle) significantly decreased from 95.40 (greenish) to 65.00 (yellow-orange-reddish) in cv. Willard. Mangoes ripened at 30^{o}C (67.70) showed more pronounced variation of h^o than mangoes ripened at 20^{o}C (80.00) at day 6 of ripening whilst temperature variation of 30 to 20^{o}C showed significantly higher decrease of h^o value than 20-30^{o}C. However, h^o value was higher in cv. Karutha Colomban (118.30) than other cultivars tested and significantly decreased during ripening at different temperatures. Colour parameters L* (luminosity) and C* (chroma) increased significantly in cvs. Willard and Karutha Colomban. Temperature variations (20 to 30^{o}C and 30 to 20^{o}C) during ripening showed more distinct increase in L* and C* values, however mango fruit ripened at 30^{o}C showed significantly higher C* and L* values than ripened at 20^{o}C. In general, L* and C* values of cv. Willard (L*: 51.49 and C*: 37.71) was significantly higher than in cv. Karutha Colomban (L*: 45.29 and C*: 32.26) (Table 5.2). Percentage weight loss (whole fruit) due to transpiration and respiration (not measured) was significantly increased during ripening in both cultivars and was higher in fruits ripened at 30^{o}C than 20^{o}C. Dehydration further caused shrinkage to fruit peel ripened at 30^{o}C, which was more pronounced on day 6 in cv. Willard. Though, percentage weight loss was relatively higher on day 6 in cv. Karutha

Colomban fruit ripened at 30°C than 20°C, the shrinkage on peel was not prominent, but some fruits started to decay at the stem end (Figure 5.1). TSS was positively correlated with percentage weight loss (r = 0.73), total carotenoids, fructose, sucrose and total measured sugars, but there was no strong correlation (Appendix D14.a and b).

Table 5.2. Variation of colour parameters (C*, L* and h^o) and TSS of mango cvs. Willard (W) and Karutha Colomban (K.C) during ripening at different temperatures *viz.* 20°C, 30°C, 20°C to 30°C, 30°C to 20°C

Ripening Temperature	Ripening period	Peel colour						TSS (%)		% weight loss (whole fruit)	
		L*		C*		h^o					
		W	K.C	W	K.C	W	K.C	W	K.C	W	K.C
Baseline	Day 0	51.49	45.29	37.71	32.26	95.40	118.30	14.93	13.50	0.00	0.00
20 → 30°C	Day 3	54.89	49.66	42.44	36.97	84.70	98.30	14.27	15.03	3.25	4.50
20 → 30°C	Day 6	57.97	52.16	53.69	43.98	78.10	88.10	16.20	13.63	11.95	10.88
30 → 20°C	Day 3	52.02	NM	45.52	NM	75.30	NM	15.00	NM	9.07	NM
30 → 20°C	Day 6	60.23	NM	61.65	NM	64.70	NM	15.05	NM	12.98	NM
20 → 20°C	Day 6	57.51	45.12	49.67	36.82	80.00	102.50	14.60	13.07	7.09	7.41
30 → 30°C	Day 6	59.55	48.64	59.80	38.67	67.70	106.40	16.60	13.10	15.73	18.60
LSD *(P = 0.05)*		5.20	2.66	6.35	5.68	16.13	6.79	3.45	4.09	1.75	3.56

NM: *not measured*

A

B

C

D

E

F

Figure 5.1. Variation of objective colour and appearance of Sri Lankan mango fruit during ripening at 20^{o}C and 30^{o}C. A: mango cv. Willard fruit before ripening (day 0), B: mango cv. Willard fruit ripened at 20^{o}C on day 6, C: mango cv. Willard fruit ripened at 30^{o}C on day 6, D: mango cv. Karutha Colomban fruit before ripening (day 0), E: mango cv. Karutha Colomban fruit ripened at 20^{o}C on day 6, F: mango cv. Karutha Colomban fruit ripened at 30^{o}C on day 6

5.3.2 Sugars

Sucrose was the prominent sugar in ripe mango fruits tested and contributed 59 to 73% in total measured sugar concentration of cv. Willard and 75 to 80.59% in cv. Karutha Colomban followed by fructose and glucose. Glucose concentration was about two-fold lower in cv. Karutha Colomban than cv. Willard. Sucrose and fructose concentration increased significantly during ripening in both peel and pulp, however glucose concentration decreased drastically. The decrease of glucose was about three- to four-fold higher in fruits ripened at 30^{o}C than at 20^{o}C for cultivars tested. Ripening at 30^{o}C showed relatively high concentration of sucrose and fructose than observed at 20^{o}C ripening in cv. Willard, but it was opposite in cv. Karutha Colomban. Temperature variation during ripening, especially initial higher temperature shock (30^{o}C) increased sugar concentration during ripening (Appendix A.5.1a; Appendix A.5.1b; Table 5.3). Higher temperature enhances the hydrolysis of sugars from the starch. Total measured sugars were negatively correlated with glucose (r = - 0.95 in Willard), but strongly correlated with sucrose, fructose and total carotenoids. In general, sugars except glucose were negatively correlated with organic acids whilst they were negatively or poorly correlated with TP and flavonoids (Appendix D13 a, b, c and d).

5.3.3 Organic acids

Citric acid was prominent and contributed about 73.50% and 95% to total measured organic acid content in cvs. Willard and Karutha Colomban, respectively. AsA (6.33 mg g^{-1} DW), malic (17.37 mg g^{-1} DW) and tartaric acids (16.78 mg g^{-1} DW) were also quantified in both pulp and peel of cv. Willard, but concentration of malic and tartaric acids were eight- to ten-fold lower in peel of cv. Karutha Colomban. AsA content of cv. Karutha Colomban was mostly below the detectable limit. In general, organic acid content was significantly higher in pulp than peel and decreased during ripening. In cv. Willard, decrease of organic acids was significantly higher in mangoes ripened at 30^{o}C than 20^{o}C, but no significant variation was observed in organic acid concentration of cv. Karutha Colomban ripened at 20^{o} and 30^{o}C (Appendix A.5.2a; Appendix 5.2b; Table 5.4a and 5.4b). Total measured organic acids were negatively correlated with sugars except for glucose whilst it strongly correlated with AsA in peel (r = 0.89) and citric acid in pulp (r = 0.96) of cv. Willard. In general, organic acids were poorly or negatively correlated with total carotenoids, TP and flavonoids (Appendix D13 a, b, c and d).

5.3.4 Total phenolics

Total phenolic concentration in peel was significantly higher than in pulp in the cultivars tested. Mango cv. Willard contained relatively higher concentration of TP in both peel and pulp than cv. Karutha Colomban, but the peel/pulp ratio of TP increased during ripening and was higher in cv. Karutha Colomban (9.73 to 18.14) than Willard (6.90 to 8.01). In general, total phenolic concentration of peel increased during ripening,

however the increase was relatively higher in mangoes ripened at 20^{o}C than 30^{o}C (Appendix A.5.3a; Appendix A.5.3b; Table 5.5). TP was poorly correlated with flavonoids in cvs. Willard and Karutha Colomban (Appendix D14a and b). In general, TP was positively correlated with sugars whilst negatively correlated with organic acids.

5.3.5 Flavonoids

Mangiferin was the prominent flavonoid in mango cultivars tested followed by quercetin 3 O-glucoside, quercetin 3 O-galactoside, Quercetin 3-rhamnoside, Quercetin and Kaemferol 3-gluctoside. Mango cv. Willard contained higher concentration of flavonoids than cv. Karutha Colomban. Flavonoid content generally increased during ripening in both cultivars, but the increase was more pronounced in cv. Willard. Mango ripened at 20^{o}C showed relatively higher content of flavonoids than fruits ripened at 30^{o}C (Appendix 5.4a; Appendix 5.4b; Table 5.5). Mangiferin was poorly or negatively correlated with other flavonoids. In general, flavonoids strongly correlated with sugars whilst negatively correlated with organic acids in peel samples (Appendix D10; D14a and b).

Table 5.3(a). Changes in sugar concentration (mg g^{-1} DW) in peel and pulp of mango cv. Willard during ripening at different temperatures *viz.* 20°C, 30°C, 20°C to 30°C and 30°C to 20°C

Ripening Temperature	Ripening period	Sucrose		Glucose		Fructose		Total measured sugars	
		Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Baseline	Day 0	202.05	339.02	72.74	98.44	88.07	134.88	362.85	572.32
20 — 30°C	Day 3	242.31	429.72	46.03	81.95	73.78	139.22	362.12	650.88
	Day 6	260.05	519.92	32.45	44.50	78.09	120.10	370.60	684.52
30 — 20°C	Day 3	259.85	462.57	48.39	73.64	85.59	63.08	393.82	599.29
	Day 6	276.67	574.70	8.15	21.05	78.55	170.42	363.36	766.17
20 — 20°C	Day 6	270.86	499.28	32.95	60.20	69.65	148.19	373.46	707.67
30 — 30°C	Day 6	271.02	549.49	6.73	17.62	92.65	185.21	370.40	752.33
LSD *(P = 0.05)*		69.26		19.22		58.44		89.27	

Table 5.3(b). Changes in sugar concentration (mg g^{-1} DW) in peel and pulp of mango cv. Karutha Colomban during ripening at different temperatures *viz.* 20°C, 30°C, 20°C to 30°C and 30°C to 20°C

Ripening Temperature	Ripening period	Sucrose		Glucose		Fructose		Total measured sugars	
		Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Baseline	Day 0	189.39	455.97	34.42	52.22	61.92	99.28	285.73	607.47
20 — 30°C	Day 3	181.35	553.51	34.26	41.54	92.01	163.12	307.62	758.17
	Day 6	180.43	580.33	3.50	8.81	62.75	90.58	246.67	679.72
20 — 20°C	Day 6	189.24	570.60	12.34	15.59	60.51	143.70	262.08	729.89
30 — 30°C	Day 6	169.57	531.85	8.17	8.80	49.55	119.31	227.29	659.95
LSD *(P = 0.05)*		74.28		20.20		59.37		93.70	

Table 5.4(a). Changes in non-volatile organic acid concentration (mg g^{-1} DW) in peel and pulp of mango cv. Willard during ripening at different temperatures *viz.* 20°C, 30°C, 20°C to 30°C and 30°C to 20°C

Ripening Temperature	Ripening period	Oxalic acid		Tartaric acid		Malic acid		Ascorbic acid		Citric acid		Total measured organic acids	
		Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Baseline	Day 0	1.61	1.10	5.10	16.78	6.50	17.37	5.00	6.33	18.80	107.67	36.80	149.27
30°C	Day 3	1.40	1.24	0.72	9.03	7.64	16.25	5.15	6.69	26.96	95.73	41.86	128.94
20	Day 6	0.63	0.80	2.97	12.22	13.92	11.04	2.75	6.90	16.30	32.58	36.56	63.53
30	Day 3	0.73	0.91	1.31	10.30	16.52	23.45	7.60	7.53	13.61	81.40	39.75	123.53
20°C	Day 6	1.17	0.59	1.74	7.74	8.78	3.21	0.78	6.71	17.55	42.79	29.50	61.10
20 — 20°C	Day 6	0.88	1.14	4.90	7.23	12.16	8.61	1.05	5.17	10.45	77.41	29.27	99.55
30 — 30°C	Day 6	0.67	0.41	1.68	9.15	4.15	25.20	0.68	3.90	20.36	13.20	27.54	51.86
LSD *(P = 0.05)*		0.47		7.90		17.80		2.47		17.05		23.66	

Table 5.4(b). Changes in non-volatile organic acid concentration (mg g^{-1} DW) in peel and pulp of mango cv. Karutha Colomban during ripening at different temperatures *viz.* 20°C, 30°C, 20°C to 30°C and 30°C to 20°C

Ripening Temperature	Ripening period	Oxalic acid		Tartaric acid		Malic acid		Ascorbic acid		Citric acid		Total measured organic acids	
		Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Baseline	Day 0	1.53	0.21	11.37	2.18	8.59	2.00	0.00	0.80	14.07	116.41	35.56	121.60
30°C	Day 3	0.31	0.25	1.16	0.93	5.81	2.08	0.00	0.00	12.95	38.80	20.58	42.06
20	Day 6	0.30	0.65	1.05	0.81	6.55	3.02	0.43	0.00	16.67	23.69	25.00	28.17
20 — 20°C	Day 6	0.23	0.45	1.24	0.71	7.05	2.13	0.00	0.00	16.89	31.43	25.41	34.72
30 — 30°C	Day 6	0.20	1.00	1.40	1.22	10.35	3.83	0.71	0.00	20.31	29.50	32.97	35.55
LSD *(P = 0.05)*		0.77		9.14		5.56		-----		29.38		34.30	

Table 5.5. Changes in total phenolic and flavonoid contents in peel and pulp of Sri Lankan mango fruits during ripening at different temperatures *viz.* 20°C, 30°C, 20°C to 30°C and 30°C to 20°C. Whereas, Mangi: Mangiferin, Q 3-gal: Quercetin 3-galactoside, Q 3-glc: Quercetin 3-glucoside, Q 3-rha: Quercetin 3-rhamnoside, K 3-glc: Kaemferol 3-gluctoside (Appendix D 8 and D 9)

Cultivars	Ripening temperature	Ripening period (days)	Total Phenolics (mg GAE g^{-1} DW)		Flavonoids (mg g^{-1} DW)					
					Peel					
			Peel	Pulp	Mangi	Q 3-gal	Q 3-glc	Q 3-rha	K 3-glc	Quercetin
Willard	Baseline	Day 0	58.24	8.44	2.96	0.56	0.35	0.03	0.01	0.03
	20 → 30°C	Day 3	54.82	9.08	4.28	0.48	0.36	0.04	0.05	0.04
		Day 6	60.88	9.84	7.63	0.45	0.40	0.03	0.03	0.06
	30 → 20°C	Day 3	58.16	9.82	7.15	0.49	0.45	0.06	0.04	0.06
		Day 6	64.30	8.04	4.41	0.47	0.34	0.07	0.03	0.04
	20 → 20°C	Day 6	64.12	8.72	6.11	0.61	0.53	0.07	0.05	0.08
	30 → 30°C	Day 6	58.50	7.26	5.66	0.39	0.33	0.04	0.03	0.04
LSD (*P=0.05*)			11.02		4.37	0.24	0.17	0.04	0.01	0.03
Karutha Colomban	Baseline	Day 0	48.66	5.00	4.20	0.08	0.27	0.02	0.01	0.07
	20 → 30°C	Day 3	53.48	3.46	5.17	0.29	0.31	0.06	0.04	0.05
		Day 6	47.74	3.48	3.36	0.15	0.40	0.07	0.08	0.04
	20 → 20°C	Day 6	51.14	4.60	4.54	0.12	0.34	0.10	0.05	0.07
	30 → 30°C	Day 6	49.34	2.72	2.87	0.09	0.29	0.06	0.05	0.04
LSD (*P=0.05*)			13.85		4.70	0.19	0.26	0.04	0.03	0.06

5.3.6 Total carotenoids

Total carotenoid content increased significantly during ripening in both cultivars, but no significant variation was observed between temperature variations. Fruits ripened at 30^{o}C showed significantly higher content of total carotenoids than fruits ripened at 20^{o}C in the cultivars tested (Figure 5.2). Total carotenoids were strongly correlated with sugars (except glucose) whilst they were negatively correlated with organic acids and TP (Appendix D13.b and c).

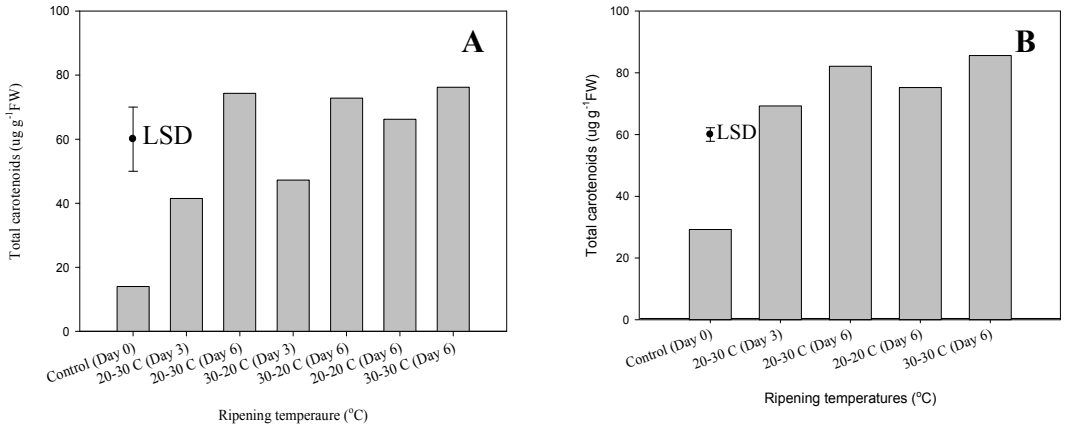


Figure 5.2. Changes in total carotenoid concentration during ripening of Sri Lankan mango (pulp) cvs. Willard (A) and Karutha Colomban (B) at different temperatures (20^{o}C and 30^{o}C)

5.3.7 Chemometric analysis

Genotypic, spatial and temporal variations in biochemical compounds during ripening at different temperatures were studied using PCA score plots, which demonstrated the clustering of samples on PC1 and PC2. Peel samples of cv. Willard were grouped away from pulp on PC1 (captured 54% of the variance), indicating that

largest contribution to biochemical variance was the tissue type. There was no distinct clustering observed between temperature variations. However, pulp showed comparatively higher temporal variation than peel (Figure 5.3a). Mango cv. Willard peel was clustered away from the cv. Karutha Colomban on PC1 (60%). However, temporal and temperature variations were higher in cv. Willard than cv. Karutha Colomban except for the baseline peel sample of cv. Karutha Colomban yet there were no distinct clustering observed (Figure 5.3b). Taste-related compounds (sugars, organic acids and phenolic compounds) of cv. Karutha Colomban also followed a similar clustering of cv. Willard, whereas peel samples were clustered away from pulp on PC1 (49%). However, there were no temporal or temperature variations in peel and pulp except the baseline pulp sample (Figure 5.3c).

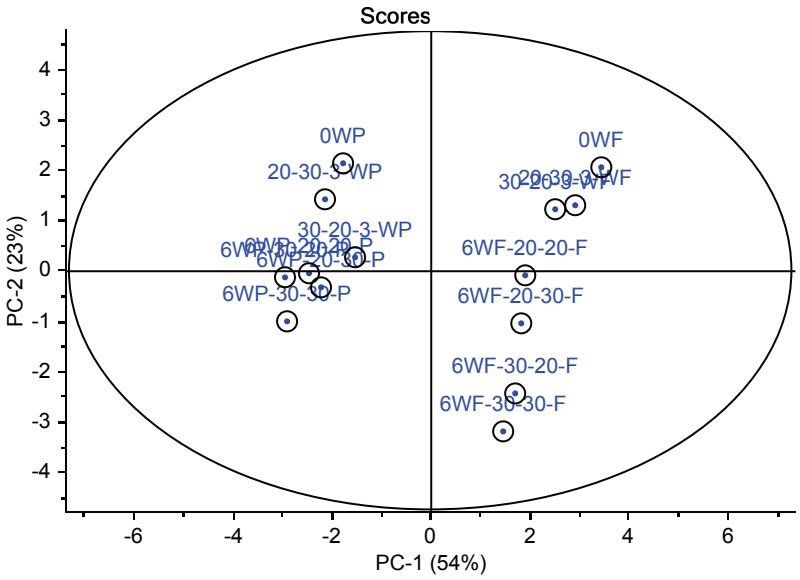


Figure 5.3(a). PCA score plot for PC1 versus PC2 of Sri Lankan mango cv. Willard (peel: WP and pulp: WF) during ripening at 32^{o}C for 4 days was performed in Unscrambler. Grouping of samples is based on similarities in spatial and temporal variation in sugars, organic acids and TP. Hotelling ellipse shows 95% confidence.

0WP: Willard peel-baseline; 20-30-3-WP: Willard peel-20-30°C-day 3; 30-20-3-WP: Willard peel-30-20°C-day 3; 6WP-30-20-P: Willard peel-30-20°C-day 6, 6WP-20-30-P: Willard peel-20-30°C-day 6; 6WP-30-30-P: Willard peel-30-30°C-day 6; 6WP-20-20-P: Willard peel-20-20°C-day 6; 0WF: Willard pulp-baseline; 20-30-3-WF: Willard pulp-20-30°C-day 3; 30-20-3-WF: Willard pulp-30-20°C-day 3; 6WF-30-20-F: Willard pulp-30-20°C-day 6, 6WF-20-30-F: Willard pulp-20-30°C-day 6; 6WF-30-30-F: Willard pulp-30-30°C-day 6; 6WF-20-20-F: Willard pulp-20-20°C-day 6

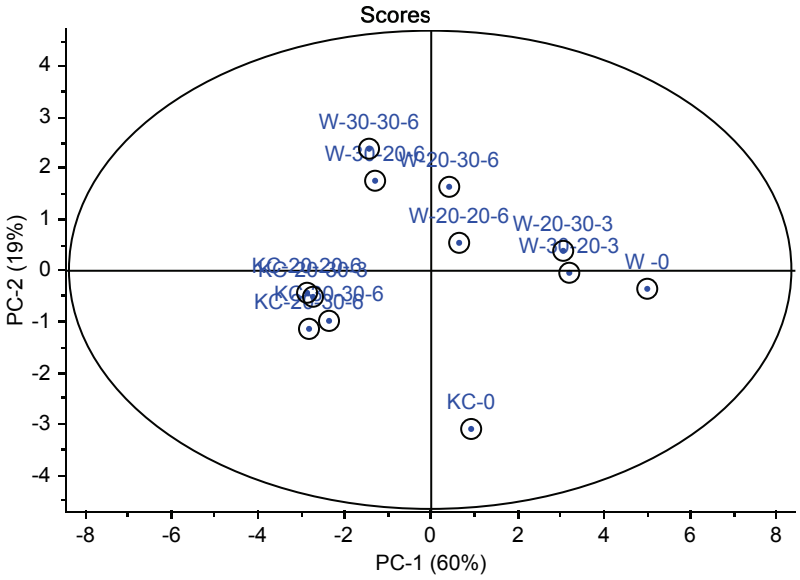


Figure 5.3(b). PCA score plot for PC1 versus PC2 of Sri Lankan mango cultivars i.e Willard (W) and Karutha Colomban (KC) during ripening at 32°C for 4 days was performed in Unscrambler. Grouping of samples is based on similarities in spatial, temporal and genotypic variation in TP, flavonoids, AsA and total carotenoids. Hotelling ellipse shows 95% confidence. W-0: Willard-baseline; W-20-30-3: Willard-20-30°C-day 3; W-30-20-3: Willard-30-20°C-day 3; W-30-30-6: Willard-30-30°C-day 6; W-20-20-6: Willard-20-20°C-day 6; W-20-30-6: Willard-20-30°C-day 6; W-30-20-6: Willard-30-20°C-day 6; KC-0: Karutha Colomban-baseline; KC-20-30-3: Karutha

Colomban-20-30°C-day 3; KC-30-30-6: Karutha Colomban-30-30°C-day 6; KC-20-30-6: Karutha Colomban-20-30°C-day 6; KC-20-20-6: Karutha Colomban-20-20°C-day 6

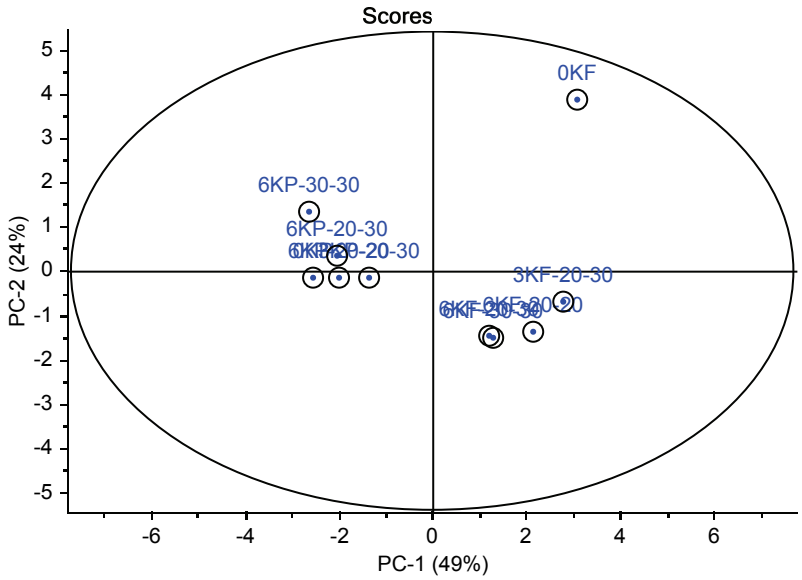


Figure 5.3(c). PCA score plot for PC1 versus PC2 of Sri Lankan mango cv. Karutha Colomban (peel: KP and pulp: KF) during ripening at 32°C for 4 days was performed in Unscrambler. Grouping of samples is based on similarities in spatial and temporal variations in sugars, organic acids and TP. Hotelling ellipse shows 95% confidence. 0KF: Karutha Colomban pulp-baseline; 3KF-20-30: Karutha Colomban pulp-20-30°C-day 3; 6KF-20-30: Karutha Colomban pulp-20-30°C-day 6; 6KF-20-20: Karutha Colomban pulp-20-20°C-day 6; 6KF-30-30: Karutha Colomban pulp-30-30°C-day 6; 0KP: Karutha Colomban peel-baseline; 3KP-20-30: Karutha Colomban peel-20-30°C-day 3; 6KP-20-30: Karutha Colomban peel-20-30°C-day 6; 6KP-20-20: Karutha Colomban peel-20-20°C-day 6; 6KP-30-30: Karutha Colomban peel-30-30°C-day 6

5.4 Discussion

Total soluble solids increased during ripening, but there was no significant difference between high and lower temperature ripening. Temperature accelerates the ripening process since it increases sugar and total carotenoid concentrations whilst decreases organic acids and phenolic acids this may be the reason for the increase in TSS. A similar variation of TSS was observed in cv. Alphonso and another seven mango cultivars during ripening at 18 to 34^{o}C (Manzano *et al*., 1997; Doreyappy-Gowda and Huddar, 2001). The TSS of cv. Kent is comparable with cultivars tested and increases up to 14.5% at day 7 of storage at 23^{o}C and then remain unchanged during further storage until day 16 (Tovar *et al*., 2001).

Peel and pulp colour of mango fruit changes during fruit maturation and subsequent ripening due to breakdown of chlorophyll and synthesis of carotenoids, xanthophylls and anthocyanins. Fruit colour enhancement during fruit development is an indicator for maturation and ripening (Ueda *et al*., 2000). A significant increase in C* and L* whilst a decrease in h^{o} during ripening demonstrate an improvement of colour properties in both cultivars, but it was more pronounced in cv. Willard fruits than cv. Karutha Colomban. The colour properties were more enhanced by the higher temperature ripening than lower temperature ripening. The peel and pulp colour change in mango fruit may be enhanced by the higher temperature during ripening, however the colour of mango fruit is genotypically defined. The h^{o} value of mango cv. Kent (104.6) is more or less similar to cultivars tested and decreased during ripening (Tovar *et al*., 2001). A decrease in h^{o} value is indication of changes in mango fruit colour from greenish to yellow and/or orange/reddish. The L* value increases whilst C* value drops during ripening at 23^{o}C in cv. Kent, but both C*and L* values increase in cultivars

tested and thus increase the colour purity. Therefore, attractive peel colour, size and shape of cv. Willard may attract more consumers to buy them.

A two- to three-fold higher percentage weight loss of whole fruit was observed in fruits ripened at 30^{o}C compared to those ripened at 20^{o}C, which predictably revealed that higher temperature induced dehydration of the fruits. Carrillo-Lopez *et al*. (2000) reported that percentage weight loss increases up to 10% in cv. Haden during ripening at 13^{o}C for 32 days. The increase of percentage weight loss may be associated with biochemical changes and dehydration during ripening. However, the percentage weight loss was comparatively more in cv. Karutha Colomban than in cv. Willard fruit. Higher temperature caused shrinkage and deformation of the mango peel at the end of the ripening period, which was more visible on the cv. Willard than cv. Karutha Colomban. This may be due to the high pulp to seed stone ratio of cv. Karutha Colomban or may be a function of metabolism but also of peel thickness. The higher temperature and lower relative humidity would have resulted in high vapour pressure deficit in fruits ripened at 30^{o}C which in-turn would increase the percentage weight loss and shrinkage. This was supported by Somboonkaew and Terry (2010) that higher vapour pressure deficit induces the water loss from Litchi fruit during storage. This might be further enhanced by the characteristics of peel, whereas mango cv. Willard peel is relatively thinner and softer than cv. Karutha Colomban peel. Therefore, distortion in the external appearance may reduce market acceptability and shelf-life of mango cv. Willard fruit ripened at higher temperature.

Sugars and acids confer flavour characteristics to ripe mango fruit (González-Aguliar *et al*., 2000), which play a major role in governing whether mango fruits are consumed through repeat purchase. Sucrose and fructose increased during ripening

whilst glucose decreased. The temporal changes in sugar composition were higher in fruits ripened at higher temperature than lower temperature. The depolymerisation of biochemical compounds and the respiration rate are induced during higher temperature ripening and subsequently yielded more glucose loss. This might be the reason for a two- to four-fold higher loss of glucose in mango ripened at 30^{o}C compared to 20^{o}C and also loss of sucrose and fructose in cv. Karutha Colomban ripened at 30^{o}C. Glucose concentration is generally lower in most of the commercial mango cultivars except cvs. Alphonso, however was comparable with cultivars tested (Yashoda *et al*., 2006). Genotypic variations and extraction methods (aqueous methanol based method) also influence the measured sugar concentration (Thanaraj *et al*., 2009). Decrease of citric (six-fold) and total measured acids (two-fold) of cv. Willard was significantly higher in fruits ripened at 30^{o}C than at 20^{o}C. The organic acid concentration of cv. Karutha Colomban also decreased during ripening, but there were no significant difference between ripening temperatures. This may be due to the degradation and further utilization of organic acids during the ripening process, which is resulted by the gluconeogenesis. Gluconeogenesis is enhanced by higher temperature. Therefore, the sugar/acid ratio of cv. Willard improved during higher temperature ripening, thus this regime increased the sweetness and market acceptability. But, the organic acid content of cv. Karutha Colomban was not influenced by the ripening temperature.

Mango fruit is a good source of antioxidants as they contain high levels of certain phenolic compounds (Ribeiro *et al*., 2007). Total phenolic concentration generally decreases during ripening and has a positive correlation with antioxidant capacity (Kim *et al*., 2007). In contrast, during low temperature storage, total phenolic content decreases but has no significant correlation with antioxidant capacity

(Shivashankara *et al*., 2004). In this study, TP content was higher in cv. Willard than cv. Karutha Colomban and increased slightly in fruits ripened at 20^{o}C (Table 5.4). However, flavonoid content generally increased during lower temperature ripening in cv. Willard whilst there was no marked increase in cv. Karutha Colomban, but decreased in general during higher temperature ripening. Therefore, the antioxidant capacity of cv. Willard increased during lower temperature ripening. Flavonoids are commonly dietary antioxidants and have become very popular since they have many reported health promoting effects such as anti-allergic, anti-cancer, antioxidant, anti-inflammatory and anti-viral activities. Quercetin is known for its ability to relieve hay fever, eszema, sinusitis and asthma. Flavonoids also prevent the oxidation of low-density lipoprotein thereby reducing the risk of development of atherosclerosis and heart diseases. Studies also suggest that flavonoids can protect low-density lipoprotein (LDL) from being oxidized by these two mechanisms. The contribution of flavonoids to the total antioxidant activity of components in food can be very high as the daily intake can vary between 50 to 500 mg (Anon, 2005). However, van Acker (1998) suggests that the daily intake of flavonoids may range between 50 to 850 mg. This intake is comparatively higher than average daily intake of other dietary antioxidants like vitamin C (70 mg), vitamin E (7-10 mg) or carotenoids (2-3 mg) (Buhler and Miranda, 2000).

The composition and concentration of carotenoids in mango fruit vary with cultivars, climactic effects, stage of maturity, fruit processing and storage conditions (Mercadante and Rodriquez-Amaya, 1998). Total carotenoid content of mango cultivars tested increased significantly during ripening (Figure 5.2). However, the increase was more pronounced in fruits ripened at 30^{o}C than 20^{o}C. Increase in carotenoids improves colour properties and antioxidant capacity (minor effect) of mango fruit. β-carotene is

the major carotenoid and composed of two retinyl groups, which are broken down in the mucosa of the human small intestine by β-carotene 15, 15'-monooxygenase to retinal (a form of vitamin A). The α- and γ-carotenes also have some vitamin A activity (though less than β-carotene) due to their single retinyl group (β-ionone ring). All other carotenoids, including lycopene, have no β-ring and thus no vitamin A. The degradation of chlorophyll and the synthesis of other pigments like carotenoids are enhanced by the higher temperature than lower temperature. Thus in-turn improve the colour of the fruit.

Pulp samples were clustered away from peels due to significant difference in sugars, organic acids and TP concentration. Since there were no distinct clustering within peel and pulp samples, temporal variation and ripening temperature had less variance than spatial variation. However, baseline pulp sample of cv. Karutha Colomban was clustered away from other samples as it had significantly higher content of citric acid and lower content of sucrose. Mango cv. Willard peel samples were clustered away from cv. Karutha Colomban based on the concentration of AsA, TP, flavonoids and total carotenoids, which indicated that this genotype had higher variance over temporal and temperature variation. Comparatively higher variation among peel samples of cv. Willard indicated that ripening period and temperature variations had a greater influence on health-promoting properties than for cv. Karutha Colomban. Therefore, PCA (Figures 5.3a and c) demonstrated that spatial variation was the major discriminatory factor in taste-related compounds whilst PCA (Figures 5.3 b) revealed that genotype created more variance than temporal and temperature variations for health-promoting compounds.

5.5 Conclusions

The objective colour properties, total carotenoids, TSS, percentage weight loss of whole fruit and sugars increased during higher temperature ripening than for lower temperature ripening, however the organic acid concentration generally decreased. The sugar/acid ratio increased during ripening, but the increase was relatively higher in cv. Willard than cv. Karutha Colomban fruits ripened at 30^{o}C. Total phenolics and flavonoids were higher in cv. Willard and increased during ripening regardless of the ripening temperature whilst flavonoid content reduced during higher temperature ripening in cv. Karutha Colomban. The shelf-life of mango fruit was affected during the higher temperature ripening, but this was more pronounced in cv. Willard fruit than cv. Karutha Colomban fruit (Figure 5.1). In general, temperature shock did not cause any significant impact on mango ripening. Though major biochemical compounds of cv. Willard fruits improved during higher temperature, the shelf-life and external appearance were distorted adversely, this may cause a significant impact on the quality and therefore market demand. In addition, biochemical composition or quality parameters of cv. Karutha Colomban were not significantly influenced by the ripening temperature. Therefore, it can be suggested that higher temperature ripening be recommended where local distribution is intended (e.g. intr-regional distribution in Sri Lanaka), but that lower temperature ripening of mango fruit is recommended (as far as biochemical compounds and quality parameters are concerned) when fruit are destined for consumption in markets farther a field.

CHAPTER SIX

Temporal variation of volatile compounds from Sri Lankan mango fruit during ripening at different temperatures

6.1 Introduction

Mango (*Mangifera indica* L.) is mainly consumed in tropics as a dessert for its sweetness and characteristic aroma. Volatile compounds found in mango fruits are responsible for its aroma and contribute to overall flavour (Brackmann *et al*., 1993). Lebrun *et al*. (2008) reported that a wide range of volatile compounds *viz*. monoterpenes, sesquiterpenes, esters, lactones and furanones (up to 435) have been identified in mango fruit, but terpene hydrocarbons are the most important (16-90%) volatiles. Among these, 3-carene is the predominant compound found in most cultivars and is principally responsible for the typical mango aroma whilst limonene, β-ocimene, myrcene and α-terpinolene are prominent in other cultivars (MacLeod and Pieris, 1984; MacLeod and Snyder, 1985; Maarse, 1991). For example, 3-carene is the main volatile compound in Floridian (cvs. Keitt, Kent and Tommy Atkins), Brazilian and Venezuelan mango-types, yet *cis*-ocimene and/or myrcene are responsible for the unique aromatic properties of Indian mango cv. Alphonso fruit (MacLeod and Pieris, 1984; Quijano *et al*., 2007). More than 90 mango cultivars have been grown globally; however cultivars can differ markedly in their flavour characteristics (Figure 6.1) (Pino and Mesa, 2006).

Mango fruit undergo many physiological and biochemical changes during ripening (Lalel *et al*., 2003b; Vasquez-Caicedo *et al*., 2004; Rathore *et al*., 2007). The levels and composition of volatile compounds in mango fruit depend on various factors such as genotypes, pre-harvest factors (Shibamoto and Tang, 1990), harvest maturity

(Lalel *et al*., 2003a), handling (Quijano *et al*., 2007), postharvest storage conditions (temperature, gas composition, etc.) (Lalel *et al*., 2005), postharvest treatments and processing (Lalel *et al*., 2003b; Quijano *et al*., 2007) and chilling injury (Nair *et al*., 2003). Most of the volatile compounds (terpene alcohols, nor-isoprenoid derivatives and aromatic alcohols) are glycosidically bound and are only liberated during ripening (Lebrun *et al*., 2008).

Volatile compounds that contribute to the aroma of mango fruit have been studied extensively in several cultivars; however there is a paucity of information on volatiles of mango cultivars endemic to Sri Lanka. Though steam distillation and/or solvent extraction procedures are widely employed in the extraction of volatiles in earlier studies, this method is believed to modify the flavour profile of a fruit sample quantitatively and qualitatively and is not considered appropriate for large number of samples. Therefore, researchers are now more interested in analysing volatiles using a head space (HS) sampling techniques which encompass a solid phase micro extraction (SPME) fibre since it is a solvent-free and rapid approach, where volatile compounds are trapped on a solid support (Sakho *et al.*, 1985; Bartley and Schwede, 1987; Koulibaly *et al.*, 1992). MacLeod and Pieris (1984) reported on volatile profile of Sri Lankan mango cvs. Willard, Karutha Colomban and Parrot; the volatiles were analysed using a gas chromatography-electron ionization mass spectrometry (GC-EIMS) and GC-chemical ionization mass spectrometry (GC-CIMS) techniques. Yet, the previous work by MacLeod and Pieris did not detail how volatile compounds change according to postharvest storage. Therefore, the aim of this study was to quantify the most important volatile compounds of prominent Sri Lankan mango cultivars over two harvests using a bespoke GC-FID (GC- flame ionization detector) technique facilitated

with HS-SPME and assess for the first time how postharvest storage regimes affects volatile profile.

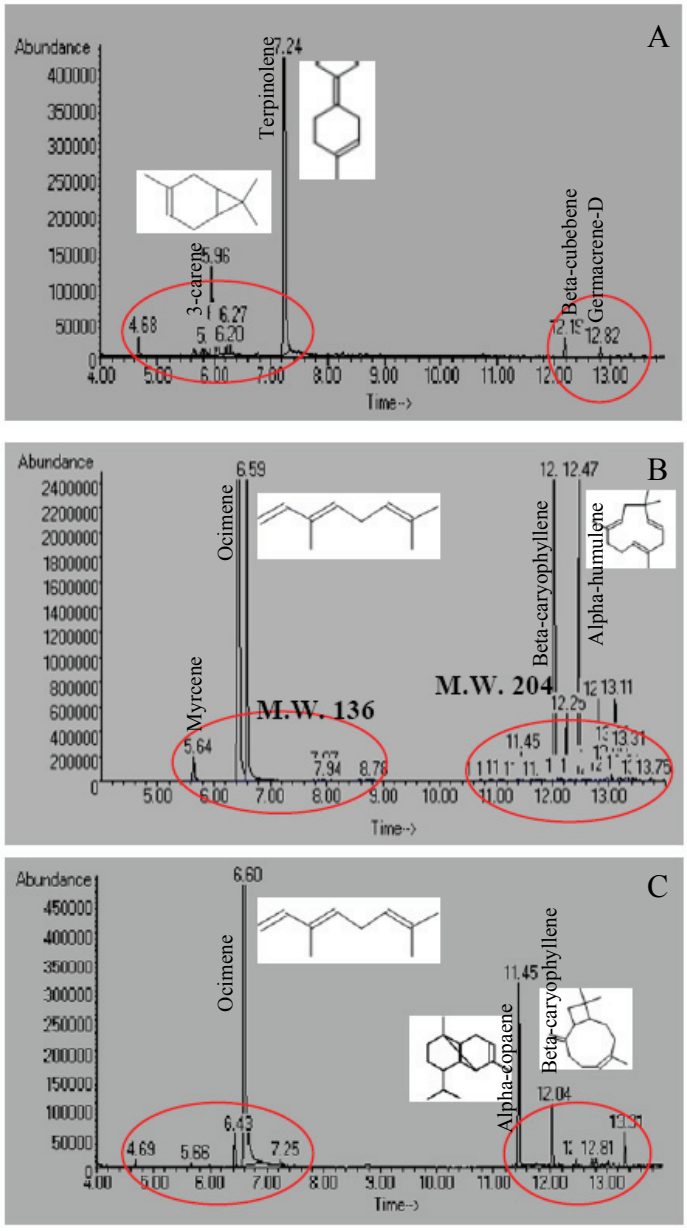


Figure 6.1. GC-MS chromatograms of head space samples of mango cvs. Aok-Rong (A), Nam-Dokmai (B) and Rad (C) (Chitsamphandhvej, 2007)

6.2 Materials and methods

6.2.1 Method development

Volatile calibration standards *viz.* α-pinene, β-pinene, 3-carene, terpinolene, β-caryophyllene, β-ocimene, myrcene, limonene, α-terpinene, α-humulene and toluene were obtained from Sigma (Sigma-Aldrich, Dorset, UK). Twenty µl of individual standards were separately mixed with 5 ml of MeOH into 20 ml glass vials (Bonosilicate, Waters) with PTFE (Polytetrafluoroethylene)-silicon septum screw caps at the concentration of 4 µl ml^{-1} and extracted using a 100 µm polydimethylsiloxane coating HS-SPME (Supelco, Bellefonte, USA) fibre for 30 min at room temperature. Upon removal from the HS, the SPME fibre was thermally desorbed in the injector of a GC-FID (Agilent Technologies, 6890N Network GC system, USA) coupled with HB5 capillary column (30 m x 0.32 mm x 0.25 µm, Agilent Technologies). A temperature program was set in the method development in order to get better peak separation within a relatively short run time. Briefly, oven temperature was maintained at 40^{o}C for 10 min and then ramped at 12.5^{o}C min^{-1} to 65^{o}C followed by 2.5^{o}C min^{-1} to 110^{o}C and 10^{o}C min^{-1} to 150^{o}C and then kept for 5 min. Detector and injector temperatures were set as 270^{o}C and 220^{o}C, respectively. Helium was used as carrier gas at a flow rate of 1 ml mim^{-1}. Retention time of individual volatile standards was recorded.

The mixed calibration standards were made by mixing a 30 µl of each standard together into a 10 ml of MeOH, a stock standard (3 µl ml^{-1}) further diluted into 1.5, 0.75, 0.3, 0.15, 0.03 and 0.015 µl ml^{-1} since mango had different volatile compounds in wide range of concentrations. Then mixed standards were extracted and analysed as previously described. The peaks of mixed calibration standards were confirmed with

peaks of individual standards according to their retention time. The calibration curve of each mixed standard was used for the quantification of volatile compounds in mango samples.

6.2.2 Optimisation of extraction procedures

A series of extraction procedures were tested on spare mango samples (cvs. Keitt and Malgova) to optimise the concentration and separation of volatiles under different extraction conditions. One g of pulp samples (cv. Keitt) was homogenised in 5 ml of saturated NaCl (pH: 5.51) and extracted using HS-SPME for 10 and 30 min at ambient temperature (23 ± 2^{o}C) in order to optimise the extraction time. Then, SPME was thermally desorbed into GC as described in section 6.2.1.

A different extraction protocol was also used in order to study the variation in the concentration of volatiles during transfer of homogenate for HS-SPME extraction. Briefly, 1 g of pulp sample was homogenised in 5 ml of saturated NaCl at ambient temperature and then the homogenate was transferred into a 20 ml vial for HS-SPME extraction. The said protocol was also practiced by Engel and Tressl (1983); Lalel *et al.* (2003b) and Dang *et al*. (2008a) in different studies.

In order to optimise the equilibrium of volatile extraction and peak separation, 5 g of spare mango pulp sample (cv. Malgova) was homogenised in 17.5 ml saturated NaCl (pH: 5.5) and extracted using a HS-SPME at different temperatures (ambient, 32^{o}C, 40^{o}C and 50^{o}C) for 10 and 30 min.

6.2.3 Experiment 1

Mango fruits from the cvs. Willard (n = 22) and Karutha Colomban (n = 15) were picked at harvest maturity from the Eastern Region of Sri Lanka (Batticaloa) and air-freighted to UK (01.07.2008) but with uncontrolled atmospheric packaging. Mangoes reached the Plant Science Laboratory, Cranfield University within four days from harvest date. Upon arrival (fruits remained at mature green stage which is recognised as a defined maturity stage in mango production), fruits were ripened at 30^{o}C and 20^{o}C for 6 days. Furthermore, fruits (3 fruits in each cultivar) were transferred from 20^{o}C to 30^{o}C and 30^{o}C to 20^{o}C after day 3 ripening in order to understand the variation in biochemical composition due to a sudden temperature change. Sampling (out-turns) was carried out at day 0, 3 and 6 during ripening (n = 3). Whole mango fruits were weighed using a precision electronic balance (Sartorius, Switzerland) before the ripening trials began and at each out-turn. Peel (15 g FW) and pulp (20 g FW) samples were taken from each fruit and immediately snap-frozen in liquid nitrogen, and stored at -40^{o}C. Ethylene levels and respiration rates were not measured as the focus of the work was simply to quantify and profile the temporal change in volatiles in different genotypes as affected by storage temperature.

6.2.4 Experiment 2

Mango fruits (15 per cultivar) from the cvs. Willard, Karutha Colomban and Malgova were picked from the Eastern Region of Sri Lanka (Batticaloa) and air-freighted to UK on 01.08.2007 as described in experiment 1. Upon arrival at the Plant

Science Laboratory, Cranfield University, fruits were ripened at 32^{o}C for 4 days and the peel and pulp samples (n = 3) were taken at day 0, 3 and 4.

6.2.5 Preparation of original sample

Sample preparation was based on Engel and Tressl (1983); Lalel *et al.* (2003b) and Dang *et al*. (2008a). Briefly, 5 g of frozen pulp and peel samples were thawed for 10 min and then separately homogenised in 17.5 ml of saturated NaCl solution in a 40 ml glass vial (VGA-090-030N, Bonosilicate, Waters) with PTFE-silicon septum screw caps at room temperature. Peel samples were treated differently since they were crushed into small particles using a pestle and mortar whilst frozen in liquid nitrogen before being adding to a saturated NaCl solution. The slurry was left on the bench for 30 minutes in order to stabilise the temperature of the slurry before being extracted using a HS-SPME fibre.

6.2.6 Extraction and quantification of volatiles from the original samples

The extraction of volatiles followed the methods of Engel and Tressl (1983); Lalel *et al.* (2003b) and Dang *et al*. (2008b) with modification. The SPME fibre was inserted at the head space of the homogenised samples and volatiles were extracted for 30 min. Upon removal, the SPME was thermally desorbed into GC as described in section 6.2.1. Volatiles were detected using FID and quantified using calibration standards.

6.2.7 Statistical and chemometric analysis

Data was subjected to analysis of variance using Genstat for Window Version 10.1.0.198 (Oslo, Norway) as described in section 3.2.6 of Chapter 3. The PCA was carried out using Unscrambler X version 10.0.1 as described in section 4.2.7 of Chapter 4.

6.3 Results

6.3.1 Method development

Mixed calibration volatile standards showed distinct and good separation. All other targeted volatiles were clustered together except β-caryophyllene and α-humulene (Figure 6.2). 3-carene was the prominent volatile compound in cv. Keitt (spare mango) pulp samples, but the concentration was higher in samples extracted for 30 min than for 10 min at ambient temperature. Furthermore, the concentration of volatiles decreased during the transfer of homogenate into another vial to aid HS-SPME extraction (Figure 6.3). Mango cv. Malgova peel sample (spare) showed a greater variation in volatile concentration during the extraction at different temperatures and durations. Samples extracted for 30 min at the ambient temperature had higher concentration of volatiles than samples extracted for 10 min. Moreover, samples extracted for 30 min at ambient temperature also contained higher volatile content than samples extracted at 50^{o}C, 40^{o}C and 32^{o}C for 30 min (Figure 6.4).

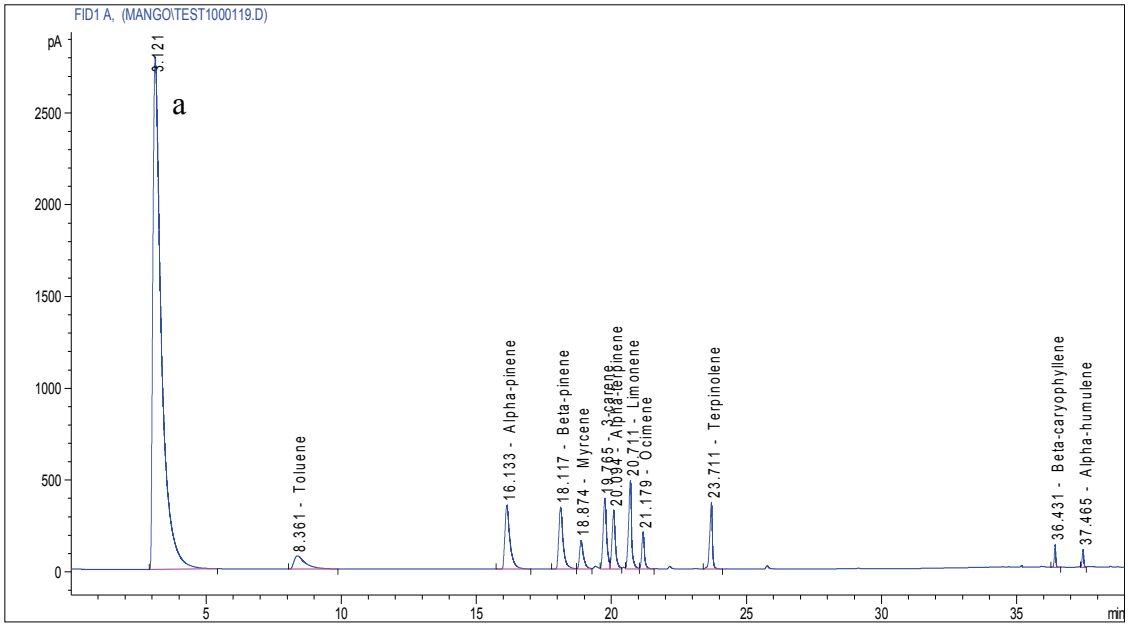
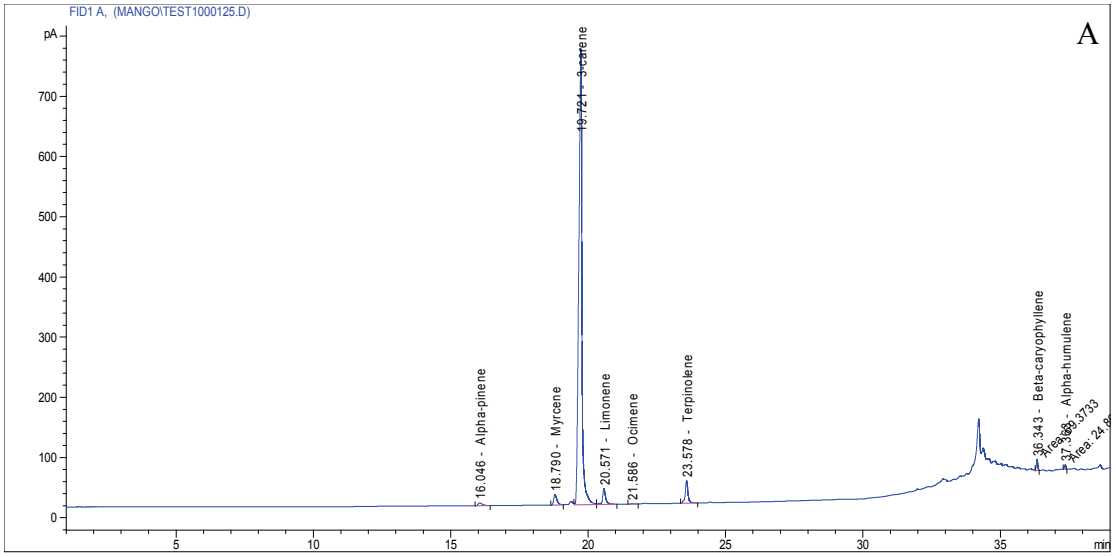


Figure 6.2. GC-FID chromatogram of mixed calibration standards of prominent volatile compounds (0.3 µl ml^{-1}). a: Methanol (solvent)



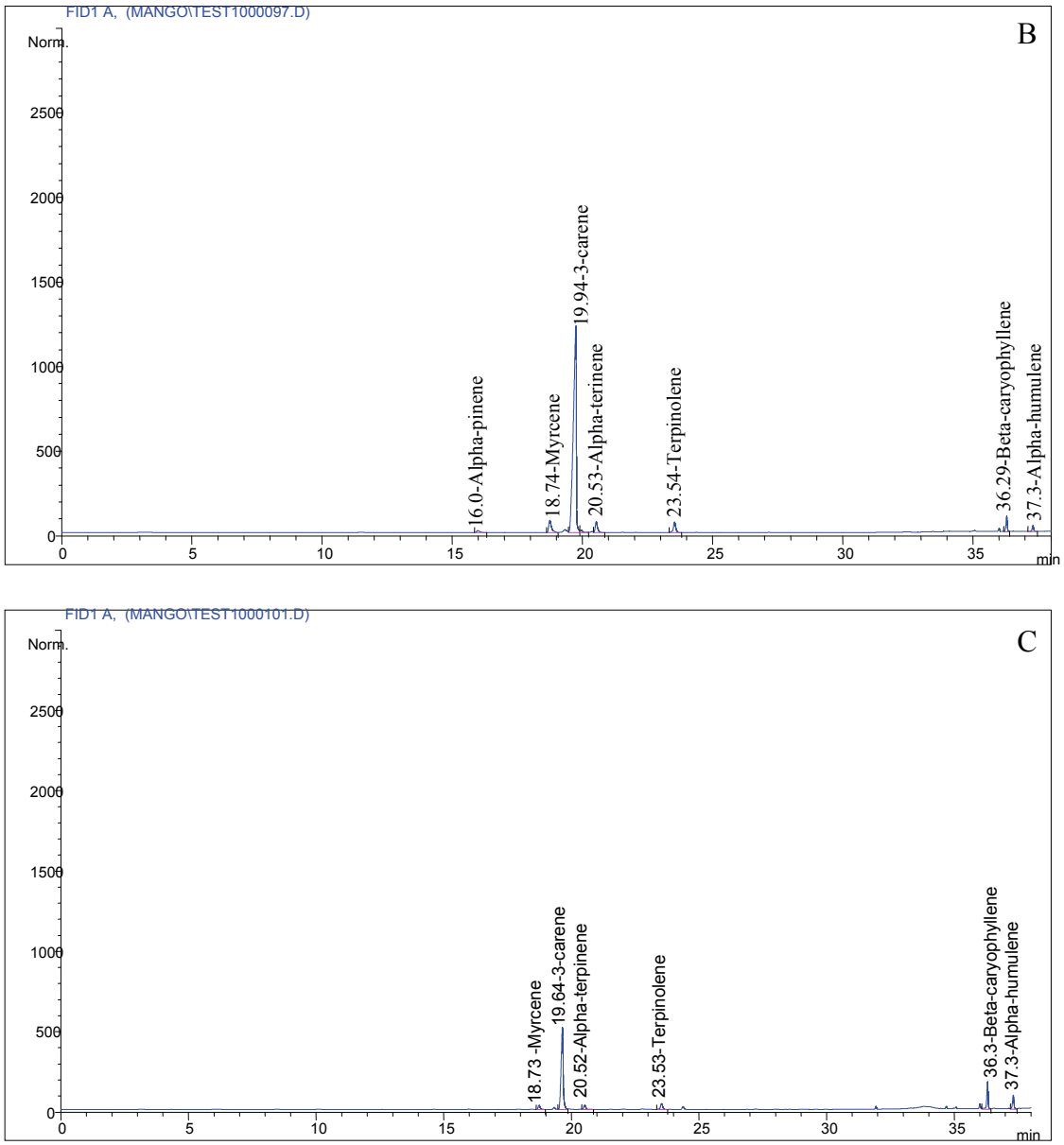
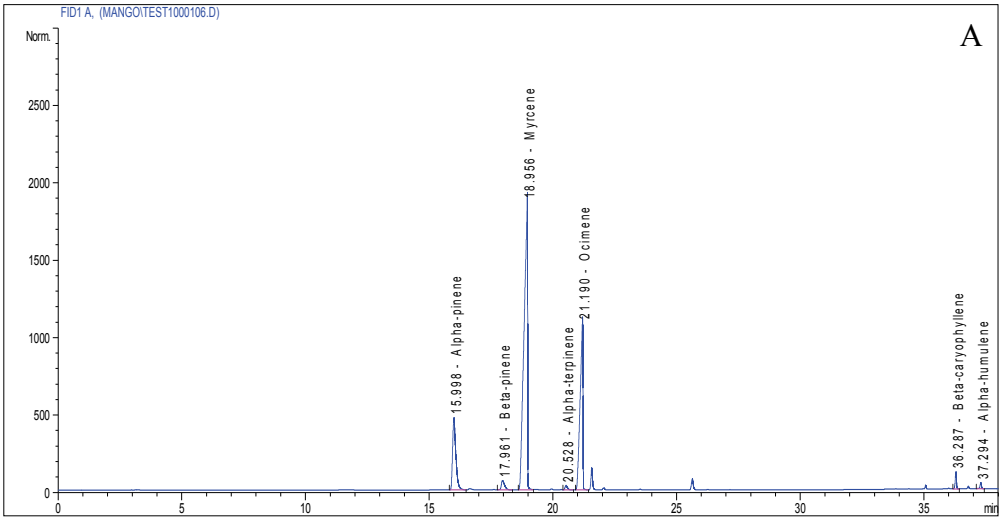
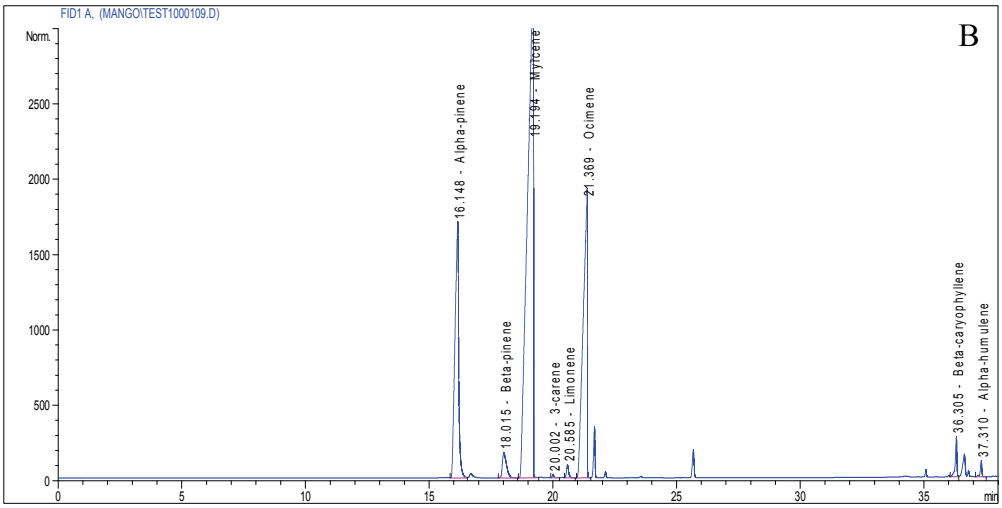
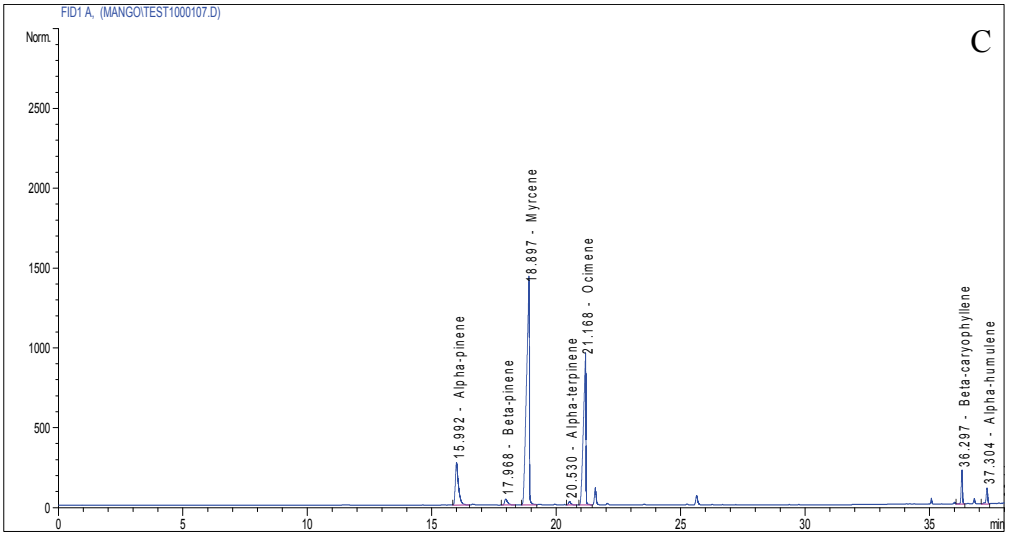


Figure 6.3. GC-FID chromatogram of spare mango cv. Keitt pulp. A: 1g sample was homogenised in 5 ml of saturated NaCl and extracted for 10 min at ambient temperature (23^{o}C). B: 1g samples was homogenised in 5 ml of saturated NaCl and extracted for 30 min at ambient temperature. C: 1 g sample was homogenised in 5 ml of saturated NaCl and then homogenate was transferred into a 20 ml vial and extracted for 10 min at ambient temperature


FID1 A, (MANGO\TEST1000106.D)
A
Norm.
15.998 - Alpha-pinene
17.961 - Beta-pinene
18.956 - Myrcene
20.528 - Alpha-terpinene
21.190 - Ocimene
36.287 - Beta-caryophyllene
37.294 - Alpha-humulene
min


FID1 A, (MANGO\TEST1000109.D)
B
Norm.
16.148 - Alpha-pinene
18.015 - Beta-pinene
19.194 - Myrcene
20.002 - 3-carene
20.585 - Limonene
21.369 - Ocimene
36.305 - Beta-caryophyllene
37.310 - Alpha-humulene
min


FID1 A, (MANGO\TEST1000107.D)
C
Norm.
15.992 - Alpha-pinene
17.968 - Beta-pinene
18.897 - Myrcene
20.530 - Alpha-terpinene
21.168 - Ocimene
36.297 - Beta-caryophyllene
37.304 - Alpha-humulene
min

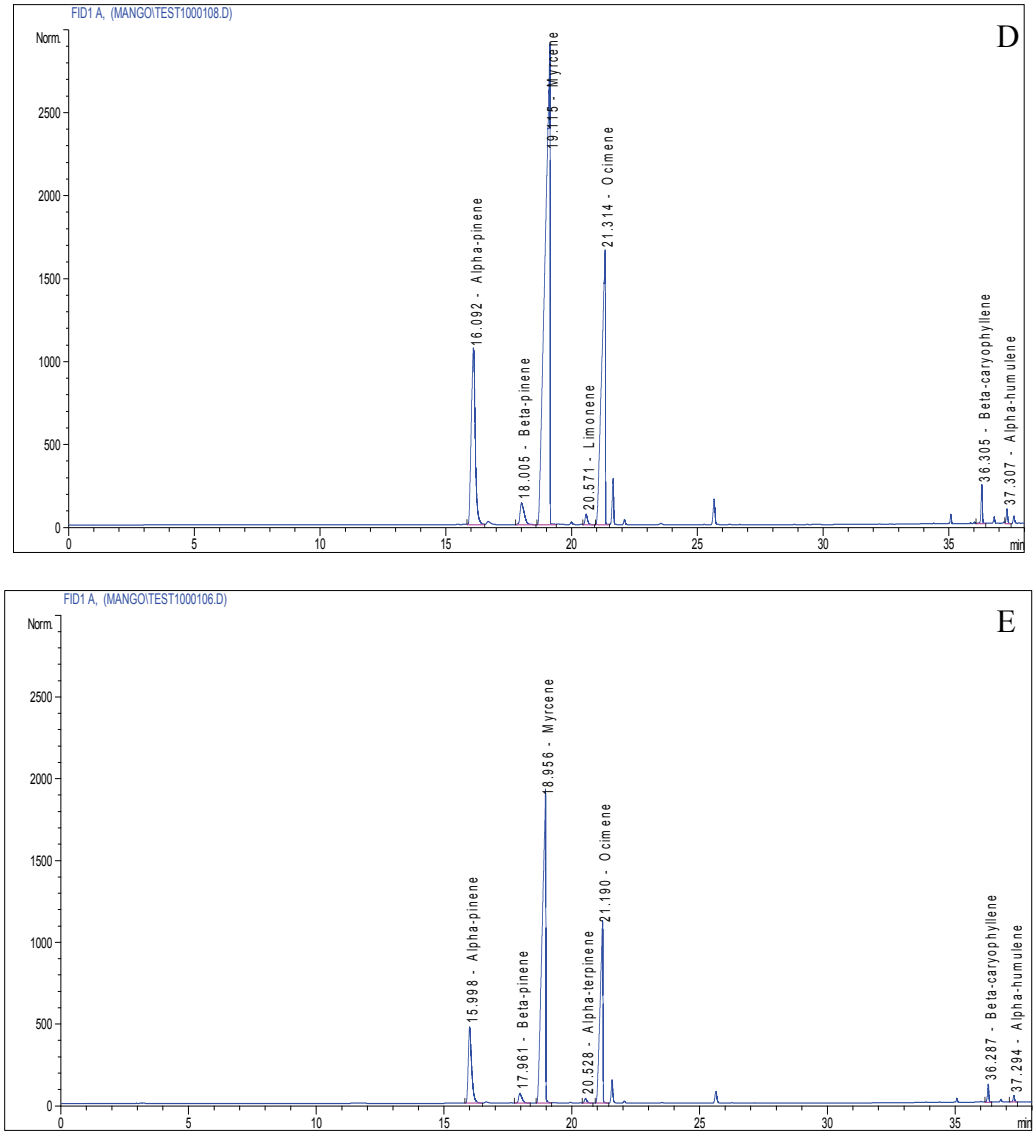


Figure 6.4. GC-FID chromatogram of volatiles in mango cv. Malgova peel. 5g of sample homogenized in 17.5 ml of saturated NaCl and extracted using HS-SPME. A: At ambient temperature (23 ± 2^{o}C) for 10 minutes. B: At ambient temperature for 30 minutes. C: At 50^{o}C for 30 minutes. D: At 32^{o}C for 30 minutes. E: At 40^{o}C for 30 minutes

6.3.2 Experiment1

Terpinolene was the prominent volatile compound measured in both peel (1849.0 μg kg^{-1} FW) and pulp (588.0 μg kg^{-1} FW) of mango cv. Willard followed by α-pinene (peel 1:1.8 and pulp 1:2), β-caryophyllene (peel 1:4.1 and pulp 1:5.4), 3-carene (peel 1:7.7 and pulp 1:8.8) and α-humulene (peel 1:7.9 and pulp 1:10),. However, other targeted volatile compounds were also found in considerable quantities (Table 6.1a) (Figure 6.5a). Generally, peel had three- to ten-fold higher concentrations of volatile compounds than pulp. The concentration of volatile compounds increased significantly in cv. Willard pulp during ripening, but the increase was higher in fruits ripened at 20^{o}C than 30^{o}C. Meanwhile, volatile concentration decreased in peel during ripening; however the decrease was higher in fruits ripened at 20^{o}C than 30^{o}C. In contrast, volatile content of cv. Karutha Colomban pulp decreased in fruits ripened at 20^{o}C, but increased by more than two-fold in fruits ripened at 30^{o}C. Mango cv. Karutha Colomban peel also showed a similar variation of volatiles during ripening, but the concentration was about three- to five-fold higher than that of cv. Willard peel. The temperature shock during ripening had a significant impact on volatile concentration of both peel and pulp of cultivars tested as it decreased the concentration markedly at the end of day 6. Ocimene was the main volatile in peel (9194.0 μg kg^{-1} FW) and pulp (1749.0 μg kg^{-1} FW) of cv. Karutha Colomban followed by β-caryophyllene (peel 1:8.3 and pulp 1:56.4) and α-humulene (peel 1:15.3 and pulp 1:92) whilst other target volatiles were measured at much lower concentrations (Table 6.1b) (Figure 6.5b). The 3-carene (important volatile compound responsible for the characteristic aroma of many commercial mango fruit) was generally lower in cultivars tested, but cv. Willard contained several folds higher content of 3-carene than cv. Karutha Colomban.

Terpinolene was strongly correlated with α-terpinene and limonene whilst negatively or poorly correlated with other volatile compounds. Strong correlation was observed among volatiles except 3-carene, α-pinene and α-terpinene (Appendix D15).

6.3.3 Experiment 2

As per the Experiment 1, terpinolene and ocimene were dominant in peel and pulp of mango cvs. Willard and Karutha Colomban, respectively. Yet, the concentration of ocimene was about two-fold lower than that previously described. However, generally the concentration of volatile compounds reduced in both peel and pulp of cv. Karutha Colomban whilst they increased in peel and reduced in pulp of cv. Willard during ripening. Myrcene was the major volatile compound in peel (1437.0 μg kg^{-1} FW) and pulp (2644.0 μg kg^{-1} FW) of cv. Malgova, but the concentration increased about five-fold in peel during ripening whilst it reduced in pulp. Ocimene (peel 1:1.8 and pulp 1:2.1), β-caryophyllene (peel 1:1 and pulp 1:11), α-pinene (peel 1:1.4 and pulp 1:1.7) and α-humulene (peel 1:1.8 and pulp 1:9.4) were also found in relatively higher concentrations in both peel and pulp of cv. Malgova than cultivars tested and followed a similar pattern to that observed in cv. Willard during ripening (Appendix A.6; Table 6.2) (Figure 6.5a, b and c). Even though, β-caryophyllene and α-humulene were not the principal volatile compounds in the cultivars tested, they were found in relatively higher concentrations with similar variation; therefore they had a positive correlation with other compounds.

In general, cv. Malgova showed higher temporal variation of volatile compounds than other cultivars tested whilst cv. Karutha Colomban demonstrated

lowest temporal variation of volatiles during ripening. The variance was most marked in pulp samples of cv. Willard than other cultivars tested.

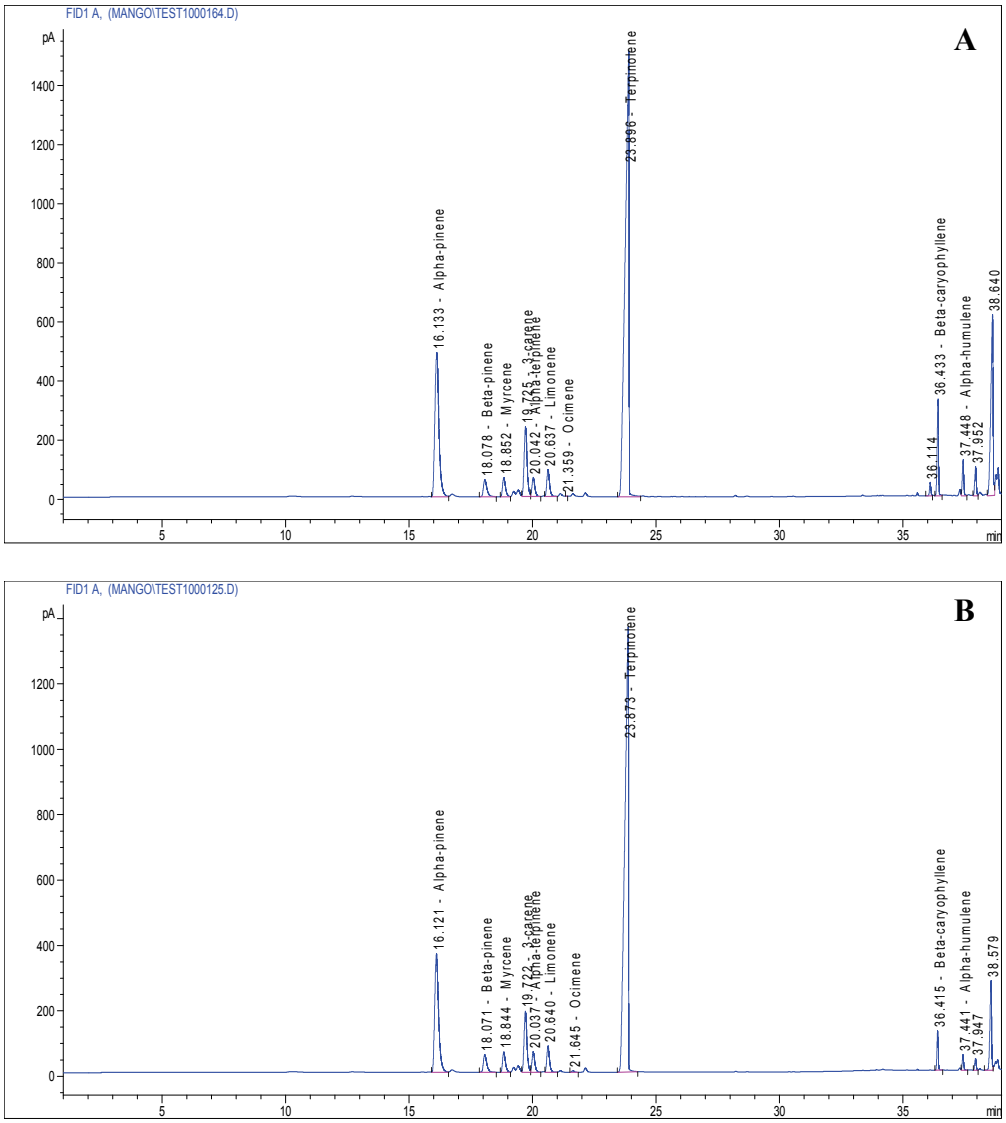


Figure 6.5(a). GC-FID chromatogram of volatile compounds in peel (A) and pulp (B) of mango cv. Willard fruit. 5g of sample homogenized in 17.5 ml of saturated NaCl and extracted using HS-SPME at room temperature (23 ± 2^{o}C) for 30 minutes

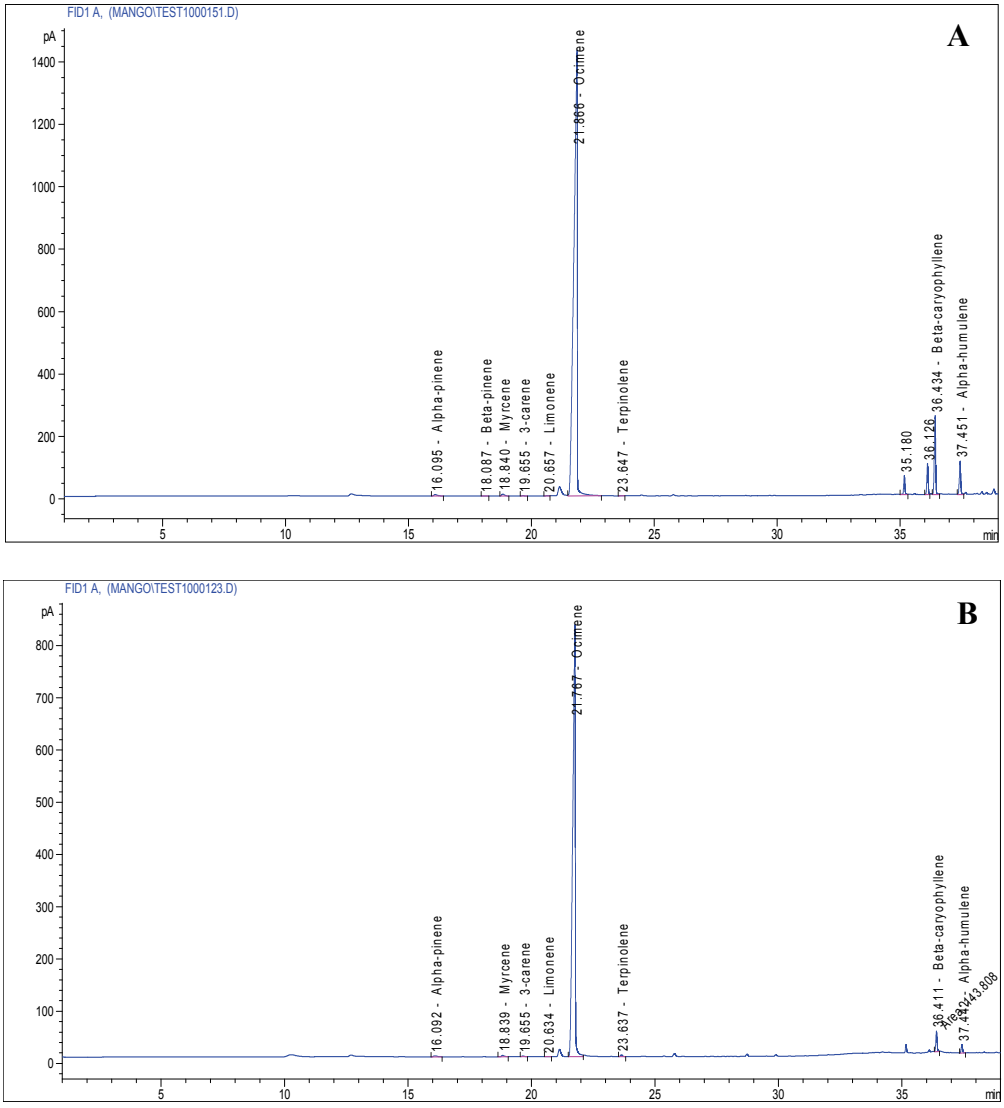


Figure 6.5(b). GC-FID chromatogram of volatile compounds in peel (A) and pulp (B) of mango cv. Karutha Colomban fruit. 5g of sample homogenized in 17.5 ml of saturated NaCl and extracted using HS-SPME at room temperature (23 ± 2^{o}C) for 30 minutes

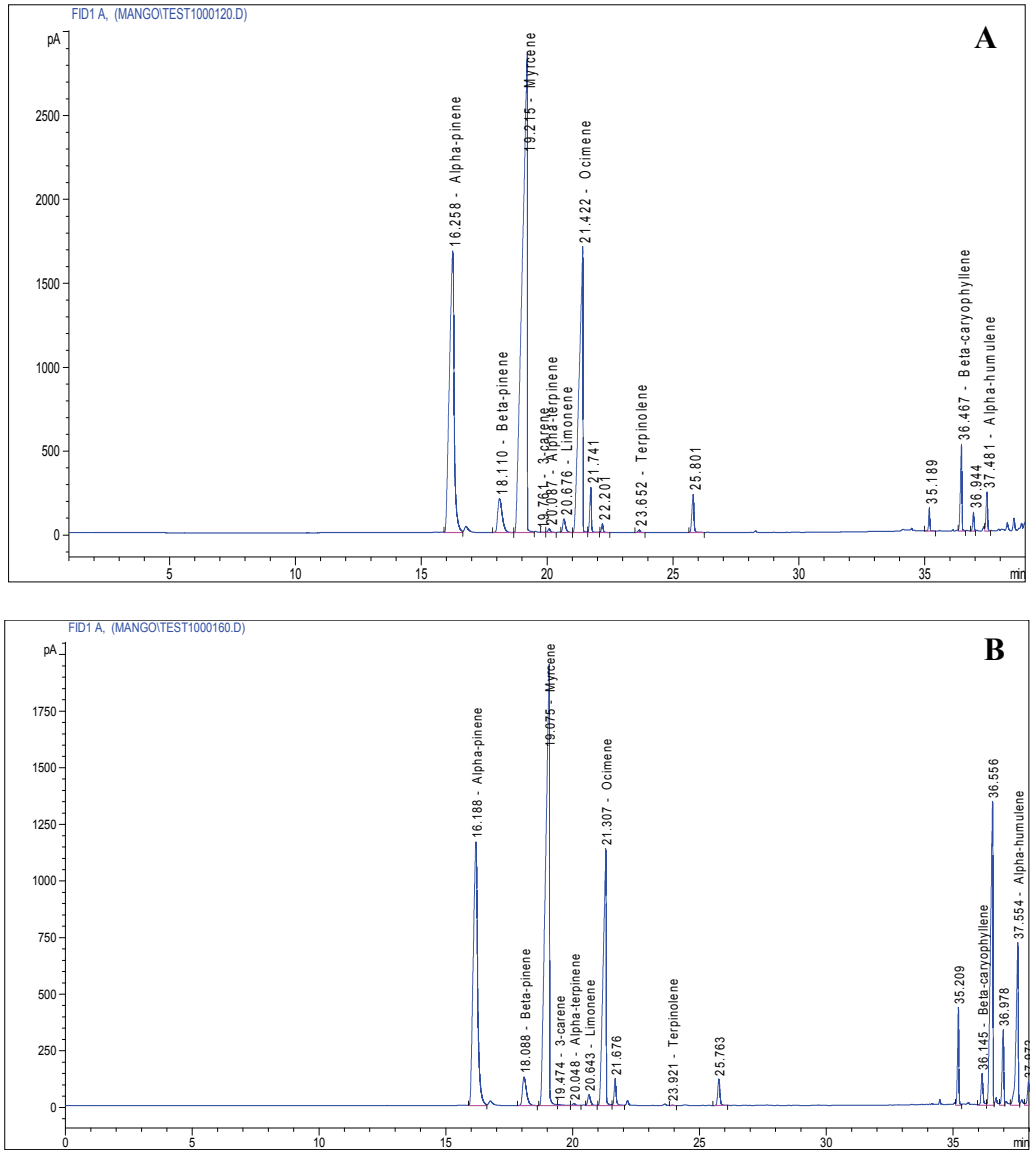


Figure 6.5(c). GC-FID chromatogram of volatile compounds in peel (A) and pulp (B) of mango cv. Malgova fruit. 5g of sample homogenized in 17.5 ml of saturated NaCl and extracted using HS-SPME at room temperature (23 ± 2^{o}C) for 30 minutes

Table 6.1(a). Variation in volatile compounds in peel and pulp of mango cv. Willard fruit during ripening at 20°C and 30°C. Mango (n=3) ripened at 20°C was transferred to 30°C whilst fruits (n=3) ripened at 30°C was transferred to 20°C at day 3. Samples were taken (n=3) before the start of ripening trial (day 0), day 3 (Temperature swap only) and day 6 from peel and pulp

Ripening Temperature	Ripe. period	α-pinene µg kg^{-1} FW		β-pinene µg kg^{-1} FW		Myrcene µg kg^{-1} FW		3-carene µg kg^{-1} FW		α -terpinene µg kg^{-1} FW		Limonene µg kg^{-1} FW		Ocimene µg kg^{-1} FW		Terpinolene µg kg^{-1} FW		β-caryophyllene µg kg^{-1} FW		α -humulene µg kg^{-1} FW	
		Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Baseline	Day 0	1025.0	300.0	141.0	36.3	108.3	39.9	241.0	67.0	2.2	33.5	85.7	1.3	12.5	4.4	1849.0	588.0	448.0	108.0	234.3	58.8
30°C	Day 3	1034.0	179.0	130.7	29.7	132.0	34.9	229.0	57.0	28.9	37.2	73.7	1.4	35.8	14.4	1622.0	622.0	298.0	23.0	163.5	10.5
20	Day 6	515.0	41.0	60.3	6.1	60.9	9.6	120.0	15.0	34.2	8.3	36.0	0.5	65.3	30.1	823.0	140.0	190.0	30.0	99.8	13.1
30	Day 3	884.0	152.0	115.1	26.8	147.4	39.3	264.0	57.0	2.9	42.3	85.4	2.0	105.2	10.8	1947.0	646.0	328.0	57.0	173.0	30.3
20°C	Day 6	647.0	104.0	67.9	16.0	69.7	31.8	138.0	48.0	32.5	29.5	4.5	1.6	40.6	22.7	798.0	601.0	197.0	113.0	100.3	66.1
20 —— 20°C	Day 6	559.0	404.0	66.1	63.9	68.4	95.2	128.0	146.0	42.5	24.9	33.2	42.6	59.0	109.0	879.0	1492.0	181.0	141.0	109.1	77.2
30 —— 30°C	Day 6	630.0	100.0	71.3	15.6	84.4	29.2	196.0	56.0	9.7	33.5	50.1	1.3	116.1	42.6	1339.0	709.0	210.0	46.0	111.3	25.0
LSD *(P = 0.05)*		NS		NS		NS		NS		NS		NS		NS		311.90		NS		NS	

NS: *not significant*

Table 6.1(b). Variation in volatile compounds in peel and pulp of mango cv. Karutha Colomban fruit during ripening at 20°C and 30°C. Mango (n=3) ripened at 20°C was transferred to 30°C at day 3. Samples were taken (n=3) before the start of ripening trial (day 0), day 3 (Temperature swap only) and day 6 from peel and pulp

Ripening Temperature	Ripe. period	α-pinene µg kg^{-1} FW		β-pinene µg kg^{-1} FW		Myrcene µg kg^{-1} FW		3-carene µg kg^{-1} FW		α -terpinene µg kg^{-1} FW		Limonene µg kg^{-1} FW		Ocimene µg kg^{-1} FW		Terpinolene µg kg^{-1} FW		β-caryophyllene µg kg^{-1} FW		α -humulene µg kg^{-1} FW	
		Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Baseline	Day 0	27.6	4.1	3.1	0.0	24.8	4.4	10.3	3.4	2.3	0.3	34.6	12.6	9194.0	1749.0	3.5	0.2	1104.0	31.0	602.0	19.0
30°C	Day 3	100.5	1.9	4.3	0.0	19.5	1.1	19.6	6.5	4.2	0.0	27.3	3.0	5422.0	442.0	1.2	0.2	479.0	11.0	269.0	6.0
20	Day 6	1.1	0.0	60.3	6.1	1.4	1.2	5.3	5.1	0.3	0.0	4.3	1.7	562.0	253.0	0.3	0.0	209.0	10.0	113.0	6.0
20 —— 20°C	Day 6	8.6	0.7	0.0	0.0	7.7	1.3	51.7	3.1	0.4	0.2	14.3	4.1	3178.0	617.0	0.5	0.4	703.0	37.0	415.0	18.0
30 —— 30°C	Day 6	10.1	3.3	1.4	1.5	12.3	8.8	16.6	0.9	0.7	2.1	39.3	12.3	5658.0	4155.0	0.4	0.2	474.0	54.0	252.0	31.0
LSD *(P = 0.05)*		NS		2.38		NS		NS		2.15		NS		NS		1.31		458.20		NS	

Table 6.2. Variation in volatile compounds in peel and pulp of Sri Lankan mango cultivars during ripening at 32°C. Samples were taken (n=3) before the start of ripening trial (day 0), day 3 and day 4 from peel and pulp

Cultivars	Ripening period (days)	α-pinene µg kg^{-1} FW		β-pinene µg kg^{-1} FW		Myrcene µg kg^{-1} FW		3-carene µg kg^{-1} FW		α -terpinene µg kg^{-1} FW		Limonene µg kg^{-1} FW		Ocimene µg kg^{-1} FW		Terpinolene µg kg^{-1} FW		β-caryophyllene µg kg^{-1} FW		α -humulene µg kg^{-1} FW	
		Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
Karutha	0	9.0	2.0	1.0	0.0	13.0	3.0	8.9	0.6	1.1	0.3	0.9	3.1	3954.0	1452.0	1.0	1.0	398.0	40.0	531.0	15.0
Colomban	3	41.0	6.0	3.3	0.3	19.0	3.0	0.4	0.0	0.7	0.0	1.3	0.3	3283.0	523.0	1.0	0.0	485.0	20.0	247.0	10.0
	4	7.0	4.0	1.6	0.4	11.0	8.0	0.1	0.2	0.0	0.0	0.4	2.5	2369.0	1015.0	1.0	0.0	461.0	22.0	256.0	15.0
Malgova	0	718.0	774.0	81.6	103.4	1437.0	2644.0	1.0	2.0	2.8	7.2	16.2	19.8	816.0	1292.0	0.0	4.0	1322.0	239.0	794.0	138.0
	3	2362.0	516.0	295.8	69.0	8831.0	1655.0	9.1	2.8	11.7	4.3	66.1	12.3	4221.0	664.0	1.0	1.0	2203.0	108.0	1409.0	66.0
	4	1475.0	399.0	175.1	61.7	6116.0	1963.0	9.5	2.6	12.6	3.2	39.1	12.5	4445.0	778.0	1.0	1.0	2451.0	94.0	1582.0	54.0
Willard	0	607.0	215.0	66.0	32.8	109.0	54.0	19.0	100.2	255.4	42.6	61.9	33.9	9.0	5.0	1911.0	1050.0	305.0	63.0	172.0	35.0
	3	234.0	58.0	21.9	6.2	38.0	9.0	8.9	2.1	87.9	25.4	26.5	5.7	230.0	12.0	513.0	133.0	140.0	14.0	89.0	7.0
	4	754.0	64.0	88.5	7.3	128.0	7.0	23.8	2.0	277.7	26.0	68.8	5.4	94.0	8.0	1995.0	142.0	347.0	15.0	203.0	8.0
LSD *(P = 0.05)*		475.90		62.78		1871.00		23.88		21.92		15.86		NS		311.90		266.90		220.40	

NS: *not significant*

6.3.4 Chemometric analysis

There was no distinct clustering observed among samples in PCA score plots based on variation of volatile compounds during ripening, however cv. Karutha Colomban peel samples were clustered away from cvs. Willard peels on PC1 (59% of the variance), indicating that the largest contribution to variance of volatiles was the samples type and cultivar. However, there was no distinct clustering observed between pulp samples of the said cultivars. Significant variation among samples based on temperature variation and ripening storage was demonstrated on PC1 in cv. Willard whilst the same variation was revealed on PC2 (29% of the variance) for cv. Karutha Colomban (Figure 6.6). Mango cv. Malgova peel samples were clustered away from cv. Karutha Colomban and PC1 (53% of the variance) whilst cv. Willard peels were clustered on PC2 (31% of the variance). However, there was no clustering among pulp samples in the concentration of volatile compounds based on ripening period. Significant variation was observed on PC1 within cv. Willard peel based on ripening period whilst the said variation was on PC2 in cv. Willard. Mango cv. Karutha Colomban peel also showed poor variation during ripening.

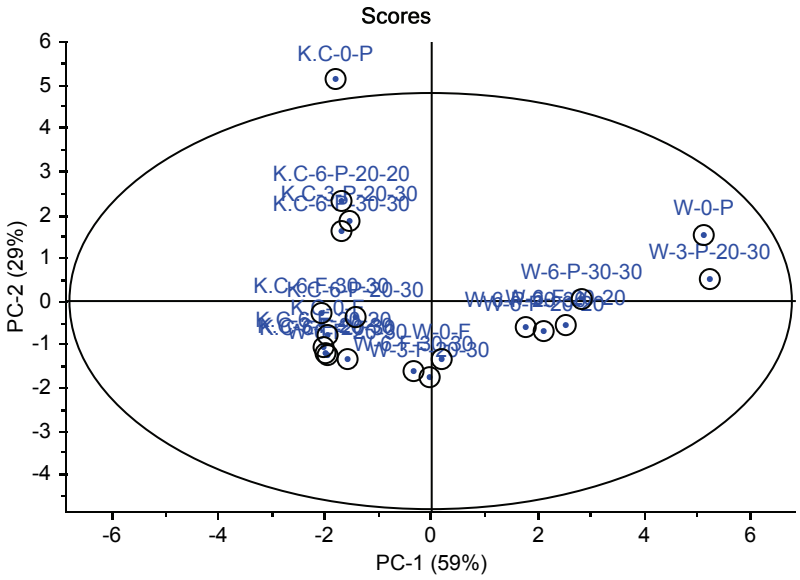


Figure 6.6. PCA score plot of mango cvs. Willard and Karutha Colomban fruit (peel and pulp) ripened at 20 and 30°C for 6 days with a temperature sock from 20 to 30°C at day 3 (Experiment 1) was performed in Genstat. Hotelling ellipse shows 95% confidence. W-0-P: Willard peel-baseline; W-3-P-20-30: Willard peel-20-30°C-day 3; W-3-P-30-20: Willard peel-30-20°C-day 3; W-6-P-20-30: Willard peel-20-30°C-day 6; W-6-P-30-20: Willard peel-20-30°C-day 6; W-6-P-20-20: Willard peel-20-20°C-day 6; W-6-P-30-30: Willard peel-30-30°C-day 6; W-0-F: Willard pulp-baseline; W-3-F-20-30: Willard pulp-20-30°C-day 3; W-3-F-30-20: Willard pulp-30-20°C-day 3; W-6-F-20-30: Willard pulp-20-30°C-day 6; W-6-F-30-20: Willard pulp-20-30°C-day 6; W-6-F-20-20: Willard pulp-20-20°C-day 6; W-6-F-30-30: Willard pulp-30-30°C-day 6; K.C-0-P: Karutha Colomban peel-baseline; K.C-3-P-20-30: Karutha Colomban peel-20-30°C-day 3; K.C-6-P-20-30: Karutha Colomban peel-20-30°C-day 6; K.C-6-P-20-20: Karutha Colomban peel-20-20°C-day 6; K.C-6-P-30-30: Karutha Colomban peel-30-30°C-day 6; K.C-0-F: Karutha Colomban pulp-baseline; K.C-3-F-20-30: Karutha Colomban pulp-20-30°C-day 3; K.C-6-F-20-30: Karutha Colomban pulp-20-30°C-day 6; K.C-6-F-20-20: Karutha Colomban pulp-20-20°C-day 6; K.C-6-F-30-30: Karutha Colomban pulp-30-30°C-day 6

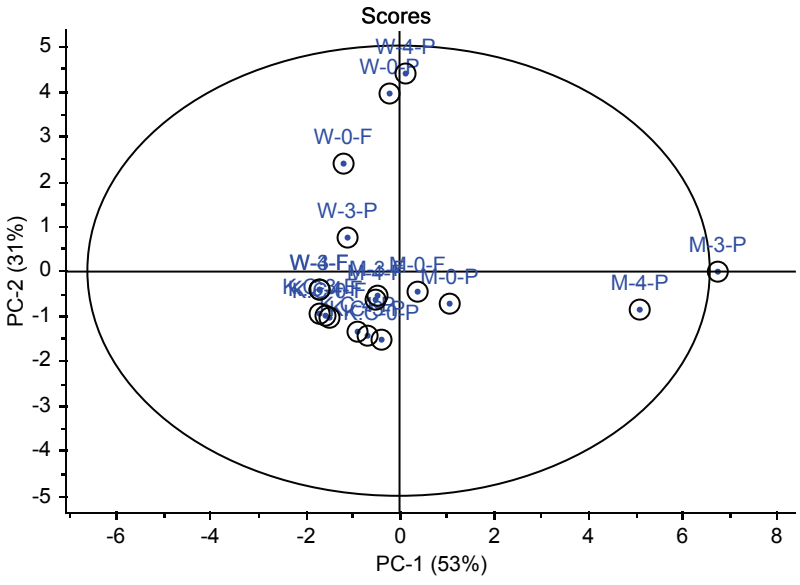


Figure 6.7. PCA score plot of mango cvs. Willard, Karutha Colomban and Malgova fruit (peel and pulp) ripened at 32°C for 4 days (Experiment 2) was performed in Genstat. Hotelling ellipse shows 95% confidence. W-0-P: Willard peel-baseline; W-3-P: Willard peel-day 3; W-4-P: Willard peel-day 4; W-0-F: Willard pulp-baseline; W-3-F: Willard pulp-day 3; W-4-F: Willard pulp-day 4; M-0-P: Malgova peel-baseline; M-3-P: Malgova peel-day 3; M-4-P: Malgova peel-day 4; M-0-F: Malgova pulp-baseline; M-3-F: Malgova pulp-day 3; M-4-F: Malgova pulp-day 4; KC-0-P: Karutha Colomban peel-baseline; KC-3-P: Karutha Colomban peel-day 3; KC-4-P: Karutha Colomban peel-day 4; KC-0-F: Karutha Colomban pulp-baseline; KC-3-F: Karutha Colomban pulp-day 3; KC-4-F: Karutha Colomban pulp-day 4

6.4 Discussion

Extraction and quantification methods significantly influenced final concentration of volatiles measured. Samples extracted for 30 min at the ambient temperature (23°C) using a HS-SPME showed higher concentration of volatiles than samples extracted for 10 min at the ambient temperature and for 30 min at 50°C, 40°C and 32°C. The analysis of volatiles using a HS-SPME coupled with GC-FID was shown to be a superior method than other methods employed in the extraction and quantification of volatiles in mango (Lalel *et al*., 2003b). The SPME is a solvent-free, rapid and sensitive technique and thus reflects the true flavour profile closely (Lalel *et al*., 2003b). Since the volatiles are trapped on a solid support in SPME, exposure on HS for longer period (30 min) facilitates SPME to reach equilibrium in the HS and trap more volatiles. Extraction temperature enhances the partitioning of volatiles into the HS, but higher temperature may alter the volatile composition.

Up to about 435 volatile compounds including monoterpenes, sequiterpenes, esters, lactones and furanones have been identified previously in various mango fruits using

different extraction and quantification methods with the major contribution being from 3-carene, limonene, β-ocimene, myrcene and α-terpinolene (MacLeod and Pieris, 1984; MacLeod and Snyder, 1985; Maarse, 1991; Nijssen *et al*., 1999; Lebrun *et al*., 2008). However, only about 40, 49 and 38 volatile compounds have been identified in Sri Lankan mango cvs. Willard, Karutha Colomban and Parrot, respectively (MacLeod and Pieris, 1984), but the majority of these compounds are in very small quantities and could not be identified properly by the mass spectra. Therefore, only the 10 most important volatiles were targeted and quantified in cultivars tested in this study using a newly developed bespoke method.

Volatiles are one of the important quality factors of fruits. The presence, abundance and the relative changes of volatile profile during ripening can determine the marketability of mango fruit (Gholap *et al.*, 1986). That said, aroma may attract more people to consume mango fruit. Pleasant and strong aroma of mango fruit also attracts seed dispersers like birds and rodents which can disperse seed far distances. Distinct aroma compounds present in significantly higher levels in mango fruit also add further value since it encourages practitioners to think about ways in which they might utilise them in the cosmetic industry and other secondary uses. Mature green (an internationally accepted and defined maturity stage in the scientific mango community) mango fruit contain trace amounts of volatile compounds, but the concentration increases during ripening as glycosidically bound volatiles are only liberated during ripening (Lalel *et al*., 2003b). Mango fruits picked at different harvest maturity show variation in volatile concentration, therefore volatiles may also be used as a maturity marker to select the fruit at optimum harvest maturity that result in better quality upon ripening (Lebrun *et al*., 2008). Mango fruit that are allowed to ripen on the tree have a different volatile profile than detached fruits that are ripened after harvest (Lebrun *et al*., 2008). That said, fruits harvested at the late maturity contain lower terpene concentration

than fruits harvested at the early maturity stage, but the concentration of esters are higher in fruits ripened on the tree (Lebrun *et al*., 2008).

Terpinolene was prominent in cv. Willard, whilst ocimene was the main compound in cv. Karutha Colomban. Myrcene, ocimene, β-caryophyllene and α-pinene were abundant in cv. Malgova (Figure 6.5a, b and c). Terpinolene has reported floral, sweet and pine-like aroma properties, whilst ocimene and myrcene are responsible for the green aroma of mango fruit (Engel and Tressl, 2002). The 3-carene (ripe mango flavour) is the most abundant free terpene found in many mango cultivars, but it may be converted into other compounds during ripening. Caryophyllene and humulene are responsible for the woody-spicy odour of mango fruit (Pino and Mesa, 2006). Therefore, higher terpinolene content of cv. Willard may enhance its flavour and appeal to some consumers. MacLeod and Pieris (1984) reported that cv. Karutha Colomban produces mainly (38%) *cis*-β-ocimene (95.1 μg kg^{-1} FW) whilst cvs. Willard (135.5 μg kg^{-1} FW) and Parrot (219.8 μg kg^{-1} FW) contains mainly terpinolene (32–35%). However, these concentrations were several-fold lower than the respective cultivars tested in this study, and thus differences may be due to extraction and quantification methods, location and mango harvesting season.

The type and concentration of volatile compounds varied greatly among cultivars tested. Volatile concentration also varied significantly within the same cultivar in the experiments conducted in this study. These differences might have been due to the varied growing location of fruit. Though fruits were picked during major harvesting season (April to July) of dry zone (Eastern region) of Sri Lanka for both experiments, the source of fruits were from different orchards in the Eastern region of Sri Lanka. Different growth locations may have differences in age of orchards, soil nutrients, source of plant stock (budded/grafted or seedling) and fruit size. Therefore, these factors may influence the type and concentration of volatile compounds. These findings are in agreement with Narain *et al*., 1998; Wilson *et*

al., 1986; MacLeod and de Troconis, 1982, where volatiles of mango cultivars vary according to location. The distinct differences among cultivars can be attributed to volatile compounds unique to each cultivar. Therefore, this is used as one of the factors to differentiate cultivars. However, mango flavour cannot be attributed specifically to any single component (MacLeod and Pieris, 1984; Lalel and Singh, 2006).

Ocimene has very strong aroma, which is the principle volatile compound in Indian mango cv. Alphonso (Selvaraj, 1989), whilst 3-carene is abundant in mango cultivars endemic to Florida (Keitt, Kent and Tommy Atkins), Brazil and Venezuela. However, 3-carene was comparatively low in Sri Lankan mango cultivars and looked more sensitive to ripening temperature since it decreased drastically during higher temperature ripening (± 30^{o}C) yet increased during lower temperature (20^{o}C) ripening (two-fold). Terpinolene content increased in cv. Willard pulp during lower temperature ripening by two- to three-fold compare to higher temperature ripening, which is in agreement with that found in cv. Kensington Pride pulp, even though terpinolene content (966.7 μg kg^{-1} FW) increased two- to three-fold (2517.2 μg kg^{-1} FW) after 3 weeks during ripening at 13^{o}C. In addition to terpinolene, 3-carene content of cv. Kensington Pride was also in line with cv. Willard and followed a similar variation during lower temperature ripening (Dang *et al*., 2008a and b). In contrast, ocimene concentration of cv. Karutha Colomban pulp increased during higher temperature ripening whilst reduced in lower temperature ripening. However, volatile concentration of peel decreased during ripening, but the decrease was higher in fruits ripened at lower temperature compare to higher temperature. This variation may be due to the changes in metabolic pathway during ripening, harvesting and postharvest storage. Factors related to species, variety and technological treatment also influence production of volatiles compounds. Aminoacid, lipid depolymerisation, shikimic acid and isoprenoid pathways play important roles in the synthesis of aroma compounds. Whereas, aldehydes and alcohols are

produced through the lipid depolymerisation pathway isoprenoids are synthesised due to the degradation of β-carotene and lycopene (Lewinsohn *et al*., 2001; Lalel *et al*., 2005; Quijano *et al*., 2007). Production of volatile compounds reduced in peel at ripe stage (the trend is vice versa in pulp) with some of the glycosidically bound volatiles being probably transported from peel to pulp as the integrity of the cell walls declined (Gomez-Lim, 1997). Therefore, it is suggested that the aroma properties of mango fruit pulp is improved during ripening due to the increased metabolism as pulp contains higher concentration of biochemical compounds than peel. The choice of ripening temperature is cultivar-oriented as far as volatiles are concerned. Acids, esters and lactones are usually developed via lipid metabolism during fruit ripening, however lipid metabolism is further enhanced by the ripening temperature. This hypothesis is supported by Lalel *et al*. (2003a) in that mango fruit (cv. Kensington Pride) ripened at 20^{o}C showed higher terpene production than fruits ripened at 15, 25, 30 and 35^{o}C. However, volatile concentration reduces during ripening at temperatures less than 13^{o}C. Variation in volatiles during higher and lower temperature ripening may be due to the inhibition of enzymes involved in several metabolic pathways (conversion of acetyl CoA to volatile compounds) and the suppression of ethylene biosynthesis (Singh *et al*., 2004). Since all other volatile compounds except ocimene were in very low concentration in cv. Karutha Colomban, ocimene could be responsible for the characteristic aroma. Relatively high variation of volatile compounds was observed during ripening of cv. Malgova whilst the variation of volatile compounds was significantly less in cv. Karutha Colomban during ripening. This revealed that volatile characteristics of cv. Malgova may be more susceptible to various factors that influence ripening i.e. temperature variation and enzyme production. Ripening of mango fruit at ± 30^{o}C showed significantly high concentration of volatiles in the cultivars tested. Peel samples of cultivars tested contained similar volatile composition, but the concentration was higher than in pulp. However, there were some definable differences

among compounds observed in both peel and pulp. Presence of higher unbound (free) volatile compounds (aldehydes, fatty acids and terpenes) in peel than pulp may be a cause for this variation. Lalel *et al*. (2003b) reported that most of the glycosidically-bound volatile compounds of cv. Kensington Pride are higher in peel than pulp at their mature and half-ripe stage than fully ripe stage. Therefore, mango peel could be utilised in pharmaceutical industry to make cosmetic products since it is not commonly consumed.

The changes in volatile production in fruits are believed to be mediated by ethylene, which is supported by the parallel production of ethylene during the synthesis of most terpenes during the ripening of mango cv. Kensington Pride (Berger, 2007). However, Lalel *et al*. (2003a) demonstrated that production of terpenes followed the ethylene production pattern in cv. Kensington Pride whilst esters continuously increased during ripening. Ethylene was not measured in the present study and so assumptions over the role of ethylene in aroma biosynthesis cannot be made. Terpenes play a major role in the characteristic aroma of mango fruit; therefore the increase in concentration of terpenes during ripening might make fruit more desirable due to enhanced flavour properties but consequently could result in greater losses by attracting more pests, diseases and rodents (Byers, 1995).

Volatile composition was the major discriminatory factor between cultivars followed by variation between peel and pulp. According to PCA score plots, genotypic variation was distinct for each cultivar tested as each contained specific volatile compounds. Peel had a higher variance than pulp since it contained significantly higher concentration of volatile compounds as compared to pulp samples with a greater variation occurring during ripening at the different temperatures tested. Baseline peel sample of cv. Karutha Colomban had very high concentration of ocimene than other samples; there it was clustered away from other samples (out of confidence ellipse). Therefore, PCA demonstrated genotypic, spatial and

temporal variations in volatile concentration during ripening, yet this was indistinct in cv. Karutha Colomban (Figure 6.7).

6.5 Conclusions

The extraction of volatiles using HS-SPME for 30 min at ambient temperature resulted in higher volatile concentrations in the cultivars tested than in commercial mango cultivars. However, the identity and concentration of volatiles varied significantly according to genotype. Tepinolene was the main volatile compound in cv. Willard whilst ocimene and myrcene were predominant in cvs. Karutha Colomban and Malgova, respectively. Therefore, it could effectively be used to differentiate mango cultivars. Furthermore, cv. Willard may be consumed by many people since it is rich in terpinolene which has an appealing scent. Peel samples contained higher content of volatiles than pulp samples. Therefore, mango peel (by product) could be utilised in the future by the pharmaceutical industry to produce cosmetic products. The influence of ripening temperature on the volatile content of mango fruit varied according to the genotypic and spatial differences. However, volatile concentration commonly increased in mango pulp during ripening whilst it decreased in peel.

CHAPTER SEVEN

General Discussion

7. 1 Introduction

Mango cultivars have been studied extensively in many mango producing countries, but there is a paucity of published information on biochemical composition of Sri Lankan mango cultivars. The commercial importance of mango fruit has increased in producing countries since the demand for mango fruit has risen around the world. However, the international trade of mango fruit is currently restricted due to the unpredictable quality and high market losses.

Mango is a climacteric fruit, which undergoes significant biochemical changes during postharvest ripening (Lalel *et al*., 2003b; Vasquez-Caicedo *et al*., 2004; Rathore *et al*., 2007). Peel and pulp colour changes to yellowish-orange due to synthesis of carotenoids and degradation of chlorophyll. Sugar content increases due to hydrolysis of starch whilst organic acid content decreases since it is broken down. Furthermore, TP and flavonoids reduce in peel but increase in pulp during ripening. However, mango fruit should be harvested at the optimum harvest maturity in order to attain better quality and nutritional composition during postharvest ripening, which might encourage greater consumption. Sugars, non-volatiles organic acids, phenolic compounds, carotenoids and volatile compounds are the major biochemical constituents in ripe mango fruit (Kauer and Kapoor, 2001). However, the type and concentration of biochemical compounds differ extensively due to genotypic variation and pre- and postharvest factors. The said compounds influence colour, aroma, taste of each cultivar. Therefore, cultivars significantly differ in these distinctive characters and become responsible for consumer acceptability (Lee and Kader, 2000). Though, more than a hundred mango cultivars are grown in different parts of the

world (Nakasone and Paull, 1998), cvs. Kensington Pride, Tommy Atkins, Haden, Kent, Keitt, Irwin, Alphonso and Uba are considered as the main commercial mango cultivars since they dominate the global market. Mangoes are, however, grown throughout Sri Lanka, yet cultivars are specific to different agro-ecological regions such that endemic cvs. Willard, Karutha Colomban and Malgova are widely distributed (Peiris and Premachandra, 2001). Mango production in Sri Lanka is seasonal, therefore surplus in-season mango fruits attain a lower price and market demand in Sri Lanka. Since prominent Sri Lankan mango cultivars (Willard and Karutha Colomban) revealed better results in terms of the quality and biochemical composition, these cultivars can be considered for the export market. However, care must be taken to adopt appropriate storage facilities (cold storage with specialised packaging, CA or MA packaging, etc.) to extend the shelf-life during long-term transportation (sea cargo shipments) whilst maintaining optimum quality.

Pre- and postharvest losses of mango fruit are comparatively high (± 40%) in Sri Lanka due to the seasonal and wide environmental variations and inadequate mechanization (Anon, 2003). The frequently experienced climatic changes in Sri Lanka cause severe pre-harvest flower and fruit drops, which subsequently impact on fruit yield in mango orchards. At its worst, only a few set fruits reach maturity stage (Chandha, 1993). Therefore, reducing the losses of mango fruit is one of the most appropriate ways to increase the total production. The extraction and quantification methods used by researchers play a significant role in determining the final concentration of biochemical composition of mango fruit and other fresh produce types. In this study, non-structural carbohydrates, non-volatile organic acids, total phenolics, flavonoids and volatile compounds of Sri Lankan mango fruits were extracted and analysed using improved methods (Appendix C). In addition, aroma volatiles were extracted and analysis in a newly developed method, therefore, this may influence concentration of biochemical compounds.

7.2 Pre-climacteric mango fruits

Sugar and organic acid concentration were generally higher in pulp than in the peel of pre-climacteric Sri Lankan mango cultivars (*viz*. Willard, Karutha Colomban, Malgova, Vellai Colomban and Ampalavi). However, there were no significant spatial and temporal variations in total phenolic content. Organic acid content decreased during maturation whilst the sugar content varied greatly in cultivars tested. This may be due to the variation in sugar and acid metabolism among genotypes. However, Saranwong *et al*. (2004) reported that sugar content increases during the maturation of Thailand mango cv. Mahajanaka. Fructose and citric acid were the most prominent sugar and organic acid in cultivars tested. In contrast, sucrose is the principle sugar in pre-climacteric mango cvs. Tommy Atkins, Delta R2E2, Baneshan, Swarnarekha, Totapuri and Kensington Pride fruit (Lima *et al*., 2001; Hymavathi and Khader, 2005; Lalel *et al*., 2005; Malik and Singh, 2006). Therefore, the pre-climacteric Sri Lankan mango fruit may be sweeter than most of the pre-climacteric commercial mango cultivars. Starch content increased during maturation in both peel and pulp of cultivars tested, whereas cv. Willard had significantly lower starch than cvs. Karutha Colomban and Malgova at the fully mature stage. However, cv. Willard had higher concentration of sugars than other cultivars tested at fully mature stage, therefore which may contribute to the final sugar content of ripe fruit. Starch is the main non-structural carbohydrate that accumulates in mature fruits and hydrolyses into sugars during subsequent postharvest ripening. Therefore, accumulating sufficient amount of starch would allow ripe fruits to assimilate a larger amount of sugars (Selvarajah *et al*., 1989; Lima *et al*., 2001). Pre-climacteric Sri Lankan mango fruit contained higher starch and lower organic acids at the fully mature stage (130 to 140 DAFB) than immature and half-mature stages. Therefore, ensuring the harvest at fully mature stage may yield better quality fruit with relatively higher nutritional composition. The starch/acid ratio increases during maturation of mango fruit

provides a better index in determining the optimum harvest maturity in pre-harvest mangoes than sugar/acid ratio (Teaotia *et al*., 1968). Mango fruits are usually harvested at an early maturity stage for export in favour of extending the shelf-life during long-term transportation. However, mango fruits harvested before the optimum harvest maturity may more likely result in poor flavour, undesirable colour and high chilling and hot water injuries (Johnson *et al*., 1997). Therefore, it can be recommended that mango fruits should be harvested at their optimum harvest maturity. The spatial distribution and temporal variation of non-structural carbohydrates and organic acids of pre-climacteric Sri Lankan mango fruit was mainly cultivar oriented and was shown for the first time in this research as not important in determining harvest maturity.

Starch content of pre-climacteric Sri Lankan mango cv. Karutha Colomban was higher than for the other cultivars compared at fully mature stage, but comparable with cv. Mahajanaka (± 55% DW). Mango cvs. Chiin Hwang No.1 (Ueda *et al*., 2001), Haden (Castrillo *et al*., 1992) and cv. Malgova were also clustered with cv. Karutha Colomban. However, Mango cvs. Willard had moderate concentration of starch (26 to 29.88% DW) and clustered with cvs. Tommy Atkins (Vergara-Valencia *et al*., 2007) and Cogshall (Lechaudel and Joas, 2006) (Appendix C). Therefore, HCA revealed geography-based clustering among cultivars except for cv. Willard. According to starch content, cv. Willard is clustered with mango cultivars commonly found in the European market (Figure 7.1). The variation in starch content may be due to the genotypic differences among cultivars yet cultivars which have high starch content may be ultimately perceived better by consumers since they would result in higher sweetness during the postharvest ripening.

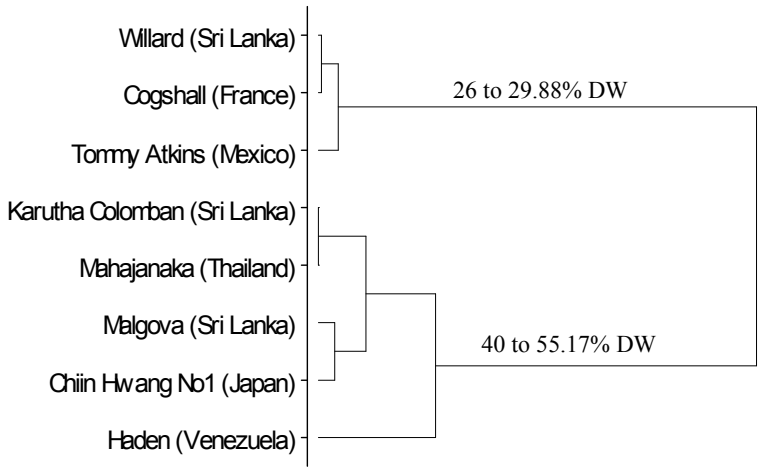


Figure 7.1. Hierarchical cluster analysis (HCA) dendrogram of starch. Clustering of mango cultivars was based on the genotypic variation of starch concentration in pulp at mature stage (defined maturity). Starch content of cultivars compared was extracted and analysed using a similar method. Starch data (Appendix C) was incorporated with the findings of this study and HCA was performed in Genstat

According to the concentration of biochemical composition at fully mature stage, mango cvs. Willard, Karutha Colomban and Malgova were considered as having potential for export market. Therefore, the said cultivars were selected for postharvest studies to understand that the spatial and temporal variations of biochemical compounds and quality parameters during ripening could be evaluated.

7.3 Postharvest mango fruit

The average ambient temperature in Sri Lanka is around 30^{o}C and mangoes are commonly ripened at ambient temperature for domestic market. Therefore, cvs. Willard, Karutha Colomban and Malgova were ripened at higher temperature (32^{o}C) to understand temporal variation of biochemical compounds and quality parameters in order to determine optimum ripening period (chapter 4). Mango fruits are commonly transported at ambient

storage (around 20^{o}C). In addition, mangoes are displayed in most of the commercial markets at around 20^{o}C and fluctuation in temperature between 20^{o}C to 30^{o}C from the point of harvest to consumption is common. In that sense, there may be a possibility for variation in biochemical compounds and quality parameters due to the temperature variation. Therefore, another study was conducted on cvs. Willard and Karutha Colomban fruits as they are considered as the most prominent dessert mango fruits in Sri Lanka. Fruits were ripened at 20^{o}C and 30^{o}C with a sudden change of temperature from 20^{o}C to 30^{o}C and 30^{o}C to 20^{o}C. Changes in concentration of biochemical compounds during low and high temperature and impact of sudden temperature change during ripening were measured (Chapter 5). Since there were no reliable methods to extract and quantify volatile compounds from mango fruit, a method was developed using GC-FID coupled with HS-SPME techniques to analyse volatiles from peel and pulp of mango fruits ripened at different ripening temperatures (32^{o}C and 20^{o}C/30^{o}C). The temporal variation and spatial distribution of volatile compounds were measured and optimum ripening period defined according to the concentration and composition of volatiles at different temperatures (Chapter 6).

Variations in concentration of biochemical compounds were observed in mango cultivars harvested during different years (2007 and 2008). Since the climatic condition was more or less similar during 2007 and 2008, orchard locations and soil condition might be the cause for those variations. Lee and Kader (2000) demonstrated that nutritional composition of a fruit vary widely based on cultivar, maturity, climate, soil type and fertility. For example, carotenoids and AsA increase with maturation and ripening conditions. AsA concentration is also influenced by the availability of the light to the crop and fruit. The concentration of starch (5%), TSS (5%), AsA (13 mg %) and total carotenoids (0.75 mg %) varied significantly in mango cv. Carabao (Philippines) fruits harvested in 1974 and 1975 due to the climatic variation between those years (Morga *et al*., 1979).

7.3.1 Total soluble solids (TSS) and percentage weight loss

Mango cvs. Karutha Colomban and Willard had higher concentration of TSS than cv. Malgova. In general, TSS increased during ripening and then decreased. This may be due to the depletion of starch reserves during the later stage of ripening, which cause a deficiency in sugars as they are utilised in respiration (Medlicott and Thompson, 1985). The TSS was higher in fruit ripened at higher temperature (± 30^{o}C) than at lower temperature ripening (20^{o}C). The increase in TSS coincides with the ripening process within the mango mesocarp, which is associated with the alteration of cell wall structure, breakdown of carbohydrates and hydrolysis of starch into sugars during ripening (Kays, 1997; Kittur *et al*., 2001). The variation in TSS is directly correlated with hydrolytic changes in starch, which is enhanced during higher temperature ripening. Percentage weight loss of whole fruit significantly increased during ripening of cultivars tested, but it was comparatively higher in cv. Karutha Colomban than for other cultivars. Fruits ripened at 30^{o}C showed higher percentage weight loss than fruits ripened at lower temperature. The changes in percentage weight loss during ripening may be due to the biochemical changes, increased respiration (not measured but assumed) and transpiration.

Total soluble solids varies considerably among mango cultivars, but TSS content of Sri Lankan mango cultivars was generally moderate (15 to 19%). Mango cvs. Cat Hoa Loc, Anwar Rotale, Faiz Kareem, Chaunsa and S. B. Chaunsa contained high TSS whilst the commercial mango cvs. Alphonso, Kent, Haden and Kensington Pride were comparable with the Sri Lankan mango cultivars tested (Manzano *et al*., 1997; Doreyappy-Gowda and Huddar, 2001; Tovar *et al*., 2001) (Appendix C). HCA clustering reflects the genotypic and geographical based variations in TSS concentration. In general, cultivars from tropical countries are clustered away from temperate cultivars apart from a few exceptions. Furthermore, cultivars from South Asian countries like India and Pakistan are further

clustered away from other tropical cultivars. Though, Sri Lanka (± 33^{o}C and 75% RH) is geographically very close to India and Pakistan, the temperature does not rise to similar heights. Therefore, Sri Lankan cultivars tended to be clustered with cultivars from moderate tropical climatic conditions (e.g. Thailand, Philippines, Japan and Mexico) (Figure 7.2).

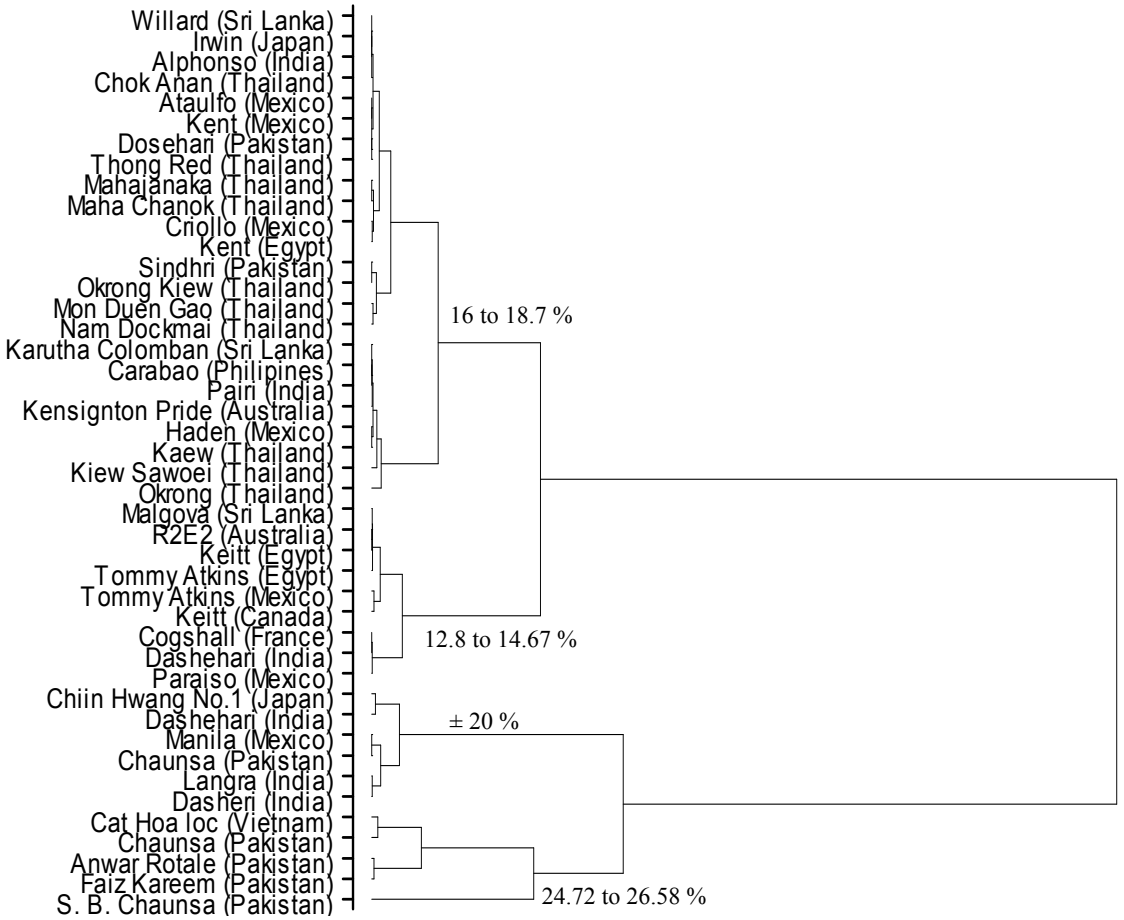


Figure 7.2. Hierarchical cluster analysis dendrogram of TSS (%). Clustering of mango cultivars was based on genotypic variation of TSS (%) in pulp at fully ripe stage. The TSS of cultivars compared was extracted and analysed using a similar method. The TSS data (Appendix C) was incorporated with the findings of this study and HCA was performed in Genstat

7.3.2 Colour and total carotenoids

Colour parameters (L*, C* and h^o) of mango fruit varied greatly during postharvest ripening. The h^o (hue angle) of mango peel reduced during ripening in cultivars tested (especially in cv. Willard), which was associated with the colour change from greenish to yellow-orange-reddish. The h^o was significantly higher in cvs. Karutha Colomban and Malgova than cv. Willard, which was evidence for their persistent greenish peel colour even after the fully ripe stage. The L* (luminosity) and C* (chroma) increased during ripening, however the values were significantly higher in cv. Willard than cvs. Karutha Colomban and Malgova. Therefore, the fruits gained attractive colour (orange-yellow) with good appearance during postharvest ripening. Predictably, colour properties of Sri Lankan mango fruits increased more during higher temperature ripening than lower temperature ripening. Higher temperature generally enhances the metabolic process such as chlorophyll degradation and carotenoids synthesis. However, the external appearance of the mango fruits (especially cv. Willard) was distorted by shrinkage at the end of the ripening period. This might be due to the transpiration of water from the fruit, higher temperature enhancing respiration and water loss, which in-turn causes fruit weight loss, shrivelling and deterioration in the appearance (Rathore *et al*., 2007). Therefore, shelf-life and quality of mango fruit was of concern during the long term higher temperature storage. The visible change in peel colour from greenish to yellow-orange was prominent during ripening at ambient temperature, however this can be delayed by lower temperature, controlled atmosphere and/or physical coatings (Yashoda *et al*., 2006; Rathore *et al*., 2007). The loss of mango peel colour (greenish) is mainly due to the degradation of chlorophyll and the synthesis of anthocyanins and accumulations of carotenoids such as β-carotene, xanthophyll esters, xanthophylls and lycopene in the plastids. Ethylene production increases after harvest and reaches climacteric peak during postharvest ripening. Ethylene induces the activity of

hydrolase enzyme which breaks down chlorophylls. Ethylene also enhances the synthesis of carotenoids, anthocyanins and xanthophylls (Yashoda *et al*., 2006; Rathore *et al*., 2007).

Mango contains substantial amounts of carotenoids, which are responsible for the yellow to orange colour in ripe fruit and substantially contributes to β-carotene supply (> 50%) mainly in tropical countries (Cano and de Ancos, 1994). Mango cvs. Willard and Karutha Colomban contained significantly high concentration of total carotenoids. Higher carotenoid content of flesh results in more attractive yellow-orange colouration which is considered desirable by consumers and indeed by seed dispersers. Carotenoid content is differentially affected by storage temperature in that total carotenoids increased significantly during ripening in the cultivars tested, yet fruits ripened at 30^{o}C showed significantly higher content of total carotenoids than fruits ripened at 20^{o}C. Total carotenoids were higher during early stage of ripening in cv. Karutha Colomban than cv. Willard.

Using HCA, mango cv. Malgova was clustered with cultivars that had less than 33 µg total carotenoids g^{-1} (FW) whilst cvs. Willard and Karutha Colomban were grouped with cvs. Kensington Pride, Sindhri and Chaunsa. Commercial mango cultivars such as Tommy Atkins, Haden, Keitt, and Palmer contained lower total carotenoids (Ribeiro *et al*., 2007; Malik and Singh, 2006) (Appendix C). Mercadante and Rodriquez-Amaya (1998) reported that composition and concentration of carotenoids varies with cultivar, climactic effects, stage of maturity, fruit processing and storage conditions. Ultimately, the results presented herein suggest that cultivars endemic to Sri Lanka should be compared to cv. Kensington Pride and other cvs. in this price bracket rather than more commercially renowned (and lower price) cultivars. Therefore, there are no distinct geographical variations observed using HCA (which only included samples for cultivars derived from tropical climate), yet climactic factors are believed to influence carotenoid content since higher temperature in

tropical countries enhances carotenoid and TSS concentration. This said HCA only included samples from cultivars derived from a tropical climate (Figure 7.3).

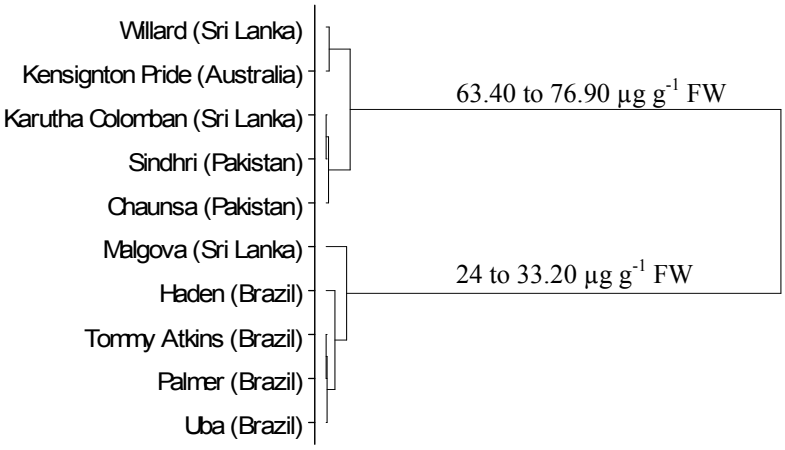


Figure 7.3. Hierarchical cluster analysis dendrogram of total carotenoids reported in mango pulp. Mango cultivars were clustered based on total carotenoid concentration in pulp at fully ripe stage. Total carotenoids of cultivars compared were extracted and analysed using a similar method. The total carotenoid data (Appendix C) was incorporated with the findings of this study and HCA was performed in Genstat

7.3.3 Sugars

The temporal change in sugars during ripening for Sri Lankan mango fruit followed that already reported for other mangoes (Castrillo *et al*., 1992). Sucrose was the main sugar in ripe mango contributing 70 to 85% of total measured sugars, followed by fructose and glucose. Glucose concentration was about ten-fold lower in cvs. Karutha Colomban and Malgova than in cv. Willard. Sucrose and fructose increased during ripening whilst glucose decreased. The increase in sugars during ripening are due to the hydrolysis of starch (Yashoda *et al*., 2006) and partial breakdown of pectins, cellulose (Roe and Bruemmer, 1981) and other polysaccharides (Kalra and Tandon, 1983). However, the decrease of

glucose was significantly greater in fruits ripened at 30°C than fruits ripened at 20°C. The marked decrease of glucose during ripening may be associated with high respiration, where glucose is used as substrate for respiration process. Glucose is oxidised into carbon dioxide, water and energy in presence of oxygen. During high respiratory period, availability of more oxygen from aerobic environment enhances catabolism of glucose. Total measured sugar content of cv. Willard fruit increased during ripening but then decreased at the end of the ripening period; this may be a result of over ripeness, which was observed in some of the fruits ripened at higher temperature. Sugar is metabolised during the maturation and ripening of mango fruit, but sugars are replenished by the hydrolysis of starch during ripening. Net sugar loss occurs during late ripening or in over ripe fruit due to the depletion of starch reserves (Medlicott and Thompson, 1985). The metabolism of sugars is enhanced by the ripening temperature. This might be the reason for two- to four-fold higher loss of glucose in mango ripened at 30°C than 20°C. The sugar content was not significantly influenced by the temperature shock.

Sri Lankan and some Pakistani mango cultivars contain higher sugar concentration (± 600 mg g^{-1} DW) compared to cvs. Kent, R2E2 (Australia) and Sindhri (Pakistan). However, cvs. Kensington Pride (Australia), Ataulfo (Mexico), Alphonso, Dashehari (India), Chiin Hwang No.1 (Japan), Cat Hoa Loc (Vietnam), Keitt (Mexico), Suwarnarekha, Baneshan (India) and Haden (Venezuela) have reportedly moderate concentration of sugars (± 450 mg g^{-1} DW) (Appendix C).

Sucrose was the prominent sugar and contributed between 60 and 85% of total sugar concentration in most of the mango cultivars evaluated in this study followed by fructose and glucose. This hierarchy of sugars levels in tissue was reflected in PCA score plots revealed whereby Sri Lankan mango cultivars together with some Pakistani cultivars were clustered away from other cultivars on PC1 (50%; captured largest variance) as they contain relatively

high sucrose (± 500 mg g^{-1} DW) and total sugar concentrations. However, Sri Lankan cultivars were grouped slightly away from Pakistani cultivars on PC2 as they are comparatively rich in reducing sugars. Mango cv. Willard was clustered away from other Sri Lankan cultivars on PC2 since it had an exceptionally high content of glucose (Figure 7.4). Indeed, sugar concentrations in Sri Lankan mango cultivars was significantly higher than most of the commercial cultivars *viz.* Kensington Pride (Malik and Singh, 2006), Keitt, Kent (Gonzalez-Aguilar *et al*., 2007), Haden (Castrillo *et al*., 1992) and Alphonso (Yashoda *et al*., 2006) cultivars tested and thus one may speculate that these fruit might be perceived by to be more sweet by consumers, although to validate this further work using trained taste panellist is required. In conclusion, PCA revealed that variation in sugar concentration mainly depends on genotype and geographical difference as most of the cultivars from South Asia were clustered away from other cultivars obtained from different parts of the world.

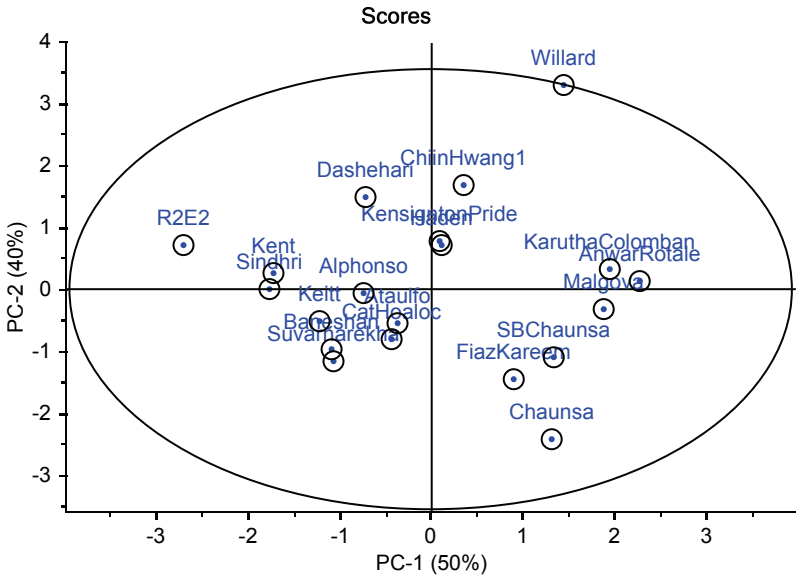


Figure 7.4. PCA score plot of sugars. Mango cultivars were clustered based on sugars concentration of pulp at fully ripe stage. Hotelling ellipse shows 95% confidence. The sugar data (Appendix C) was incorporated with the findings of this study and PCA was performed in Unscrambler.

7.3.4 Non-volatile organic acids

Citric acid was prominent in organic acid composition of cultivars tested with a considerable contribution of ascorbic, malic, oxalic and tartaric acids. Total measured organic acid concentration was significantly higher in pulp than peel and generally decreased during ripening due to the degradation and further utilization of acids in the metabolic pathway. The marked decrease in citric acid was the prime cause for the decrease in acid content during ripening, but malic and oxalic acids increased in cvs. Karutha Colomban and Willard. This variation in organic acids may be due to gluconeogenesis, which is active during the ripening. Gluconeogenesis is a metabolic pathway that results in the generation of glucose from non-carbohydrate carbon substrates such as lactate, glycerol and glucogenic amino acids and is associated with the decrease in citrate and succinate (citrate synthase activity decreases during ripening) and increase of isocitrate and succinate dehydrogenase activities (Tovar *et al*., 2001; Yashoda *et al*., 2006). A decrease of organic acid content was higher in Sri Lankan mango fruits ripened at 30^{o}C than 20^{o}C. This might be the result of organic acid metabolism, which was enhanced by the ripening temperature. Therefore, the fruit ripened at 30^{o}C may be sweeter than fruits ripened at lower temperature, but have less balanced acidity.

There was no distinct clustering observed in PCA score plot, however it did show that Sri Lankan mango cv. Malgova (94.30 mg g^{-1} DW) was clustered away from other cultivars on PC1 (54%; capturing largest variance) since it contained about three-fold higher total organic acids than cultivars tested. Therefore, cv. Malgova fruit may be preferred by few consumers as a dessert since it contains a lower sugar/acid ratio. However, cv. Malgova fruit is commonly consumed at mature stage as a pickle. Though mango cv. Willard was grouped with other cultivars, it was further clustered away on PC2 (27%) based on significantly higher content of ascorbic acid (1.3 mg g^{-1} FW) than other cultivars (Appendix

C)). Willard was the only mango cultivar which contained AsA as the second major acid in the total organic acid concentration. Therefore, genotypic variation was major discriminatory factor in organic acid content of mango fruit (Figure 7.5).

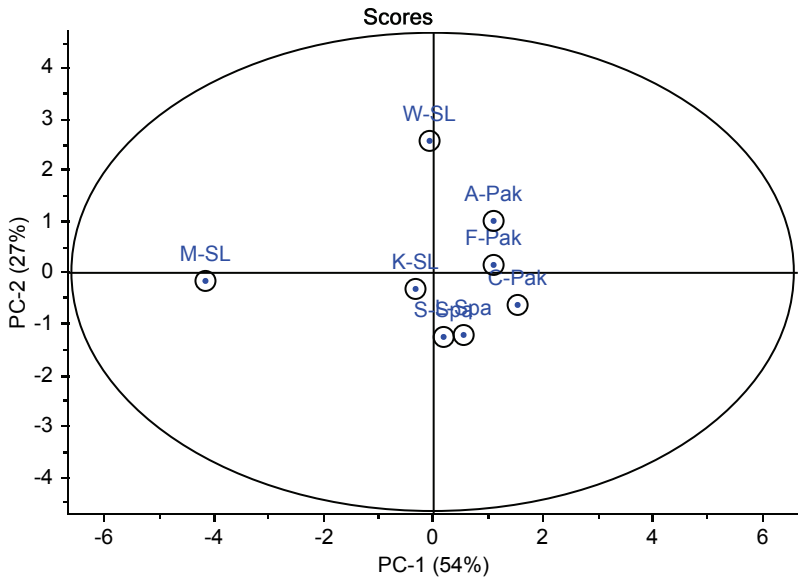


Figure 7.5. PCA score plot of non-volatile organic acids. Mango cultivars were clustered based on the concentration of non-volatile organic acids of pulp at fully ripe stage (Appendix C). Hotelling ellipse shows 95% confidence. W-SL: Willard-Sri Lanka; K-SL: Karutha Colomban-Sri Lanka; M-SL: Malgova-Sri Lanka; A-Pak: Anwer Rotale-Pakistan; F-Pak: Faiz Kareem-Pakistan; C-Pak: Chaunsa-Pakistan (Rajwana *et al*., 2010); S-Spa: Smith-Spain; L-Spa: Lippens-Spain (Cano *et al*., 1994). The non-volatile organic acid data (Appendix C) was incorporated with the findings of this study and PCA was performed in Unscrambler.

Mango cv. Willard had significantly higher content of AsA (1.3 mg g^{-1} FW) than cultivars compared and was the only mango cultivar which contained AsA as a second major acid in the total measured organic acid concentration (based on published information) (Appendix C). However, Sri Lankan mango cv. Karutha Colomban contained a moderate

content of AsA whilst cv. Malgova contained a very lower content and was comparable with most of the commercial mango cultivars *viz.* Tommy Atkins, Haden, Keitt, Kent and Palmer (Appendix C). Therefore, cultivars were clustered according to genotypic variation in AsA concentration. Since the said HCA analysis included cultivars commonly from tropical countries, climatic or geographical influences were not observed in HCA clustering. The total measured organic acid content of mango cultivars endemic to Sri Lanka may influence on consumer acceptance in the global market as it is responsible for sour taste. The sugar/acid ratio of Sri Lankan cultivars may be higher than commercial cultivars due to the relatively higher sugar content (Figure 7.6).

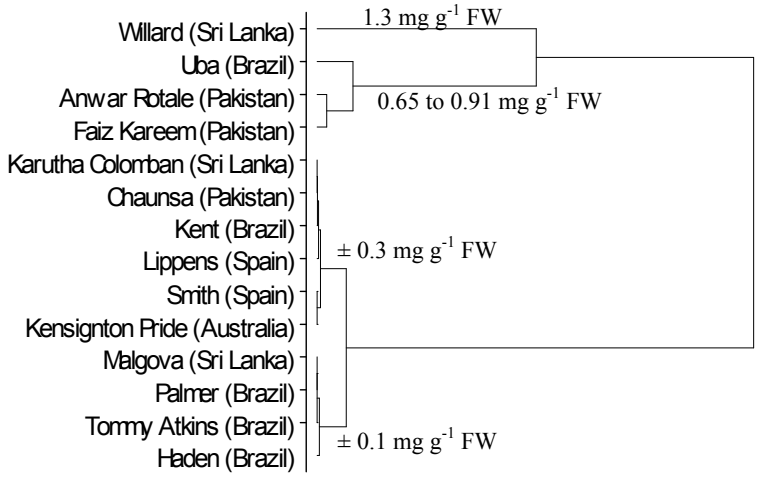


Figure 7.6. Hierarchical cluster analysis dendrogram of AsA. Clustering of mango cultivars was based on the concentration of AsA in pulp at the fully ripe stage. The AsA content of cultivars compared were extracted and analysed using a similar method. The AsA data (Appendix C) was incorporated with the findings of this study and PCA was performed in Genstat

Titratable acidity of cultivars tested decreased generally during ripening. This also further supports the increase in sugar/acid ratio during ripening in the cultivars tested; however the increase was relatively higher in cv. Willard during higher temperature

ripening. Sugars and organic acids are the major biochemical entities in fruits, mango fruit flavour and compounds associated with aroma are mainly based on the balance between sugars and organic acids (Cano *et al*., 1994). Therefore, the sugar/acid ratio is an important parameter in mango fruit as it is used to detect the harvest maturity and specific ripening behaviour; sugar/acid ratio is also decisive for basic taste and palatability of the fruits (Mahayothee *et al*., 2002). In that sense, cv. Willard may attain greater sweetness than other cultivars tested. Sri Lankan mango cvs. Malgova and Karutha Colomban had high content of titratable acidity and were clustered with cvs. Kensington Pride, Cat Hoa Loc and Sampee whilst cvs. Willard contained moderate titratable acidity (Appendix C; Figure 7.7). Therefore, HCA clustering revealed genotypic variation in TTA rather than geographical variation. However, mango fruits from tropical countries generally have more TTA than cultivars from temperate countries.

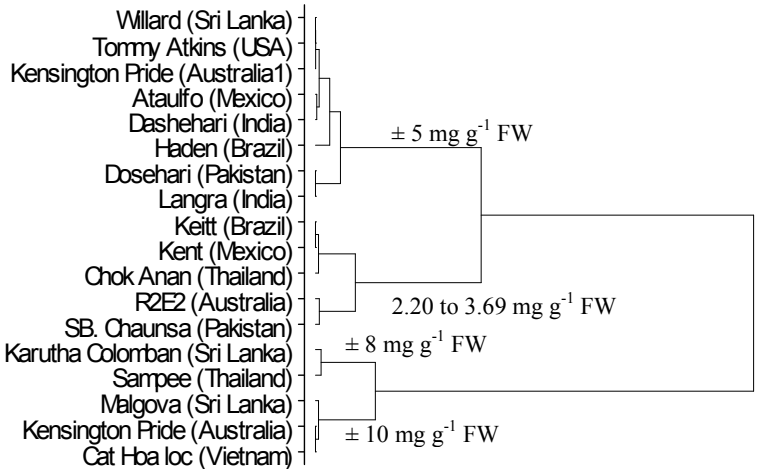


Figure 7.7. Hierarchical cluster analysis dendrogram of TTA. Clustering of mango cultivars was based on the concentration of TTA in pulp at the fully ripe stage. The TTA content of cultivars compared were extracted and analysed using a similar method. The TTA data was incorporated with the findings of this study and PCA was performed in Genstat

7.3.5 Total phenolics (TP) and flavonoids

Total phenolic concentration of cv. Willard was comparatively higher than other cultivars tested, the peel had about ten-fold higher TP than pulp. In general, TP content increased during ripening, but a marked decrease was observed in fruits ripened at 30^{o}C. Total phenolic content changed drastically between peel and pulp after harvest, whereas TP decreased in pulp after harvest but increased in peel. Therefore, the decrease in TP may improve the sweetness and colour of mango pulp, but may reduce the antioxidant capacity as it is one of important dietary antioxidant and responsible for the prevention of many chronic diseases like cancer and heart disease. Mango peel is a good source of phenolic compounds, carotenoids and other bioactive compounds (Wolfe *et al*., 2003). In order to benefit from these bioactives then peel needs to be extracted and extracts added to food or consumed as supplements.

Mango cv. Willard contained about two-fold higher content of TP in pulp (8.90 mg GAE g^{-1} DW) than cvs. Karutha Colomban and Malgova. However, other cultivars compared (Kim *et al*., 2007; Rajwana *et al*., 2010) had a very much lower content of TP in their pulp (Figure 7.8A). Mango cvs. Willard and Karutha Colomban were clustered with cv. Raspuri (Ajila *et al*., 2007a) since they contained significantly higher concentration of TP than cultivars compared in their peel (Figure 7.8B). Since a few cultivars were considered for HCA analysis mainly from South Asia, the clustering revealed mainly the genotypic variation in TP content. Total phenolic concentration of postharvest Sri Lankan mango cultivars was comparatively higher in both peel and pulp than commercial mango cultivars such as Tommy Atkins (Vergara-Valencia *et al*., 2006), Haden (Larrauri *et al*., 1996) and Uba (Ribeiro *et al*., 2007). Therefore, the cultivars tested may have high levels of individual phenolics (Appendix C).

Mangiferin was the prominent flavonoid in mango fruits (peel) tested and followed a similar variation of TP during ripening. Mango cv. Karutha Colomban contained lower content of flavonoids than cvs. Willard and Malgova. Flavonoids possess antioxidant, anticarcinogenic and antiatherogenic activities, whereas mangiferin has reported health-promoting properties *viz.* antioxidant, antitumor and antiviral activities (Ribeiro *et al*., 2008). Other quantified flavonoids were several folds lower than mangiferin, but showed a similar variation during ripening. Therefore, cvs. Willard and Malgova peel may be a potential source of antioxidants and also may gain more attention from practitioners in relation to bi-product utilization. Mangiferin was only three-fold higher in cv. Tommy Atkins peel (1690.40 µg g^{-1} DW: Berardini *et al*., 2005) than other flavonoids whilst quercetin 3 O-glucoside was the main flavonoid (370 µg g^{-1} DW) in cv. Uba (Ribeiro *et al*., 2008) followed by mangiferin (199 µg g^{-1} DW) and quercetin 3 O-glucoside. Therefore, Sri Lankan mango cultivars may have higher antioxidant capacity than cvs. Tommy Atkins and Uba. Published information on flavonoids of mango cultivars is comparatively lower than for other target analytes (Appendix C).

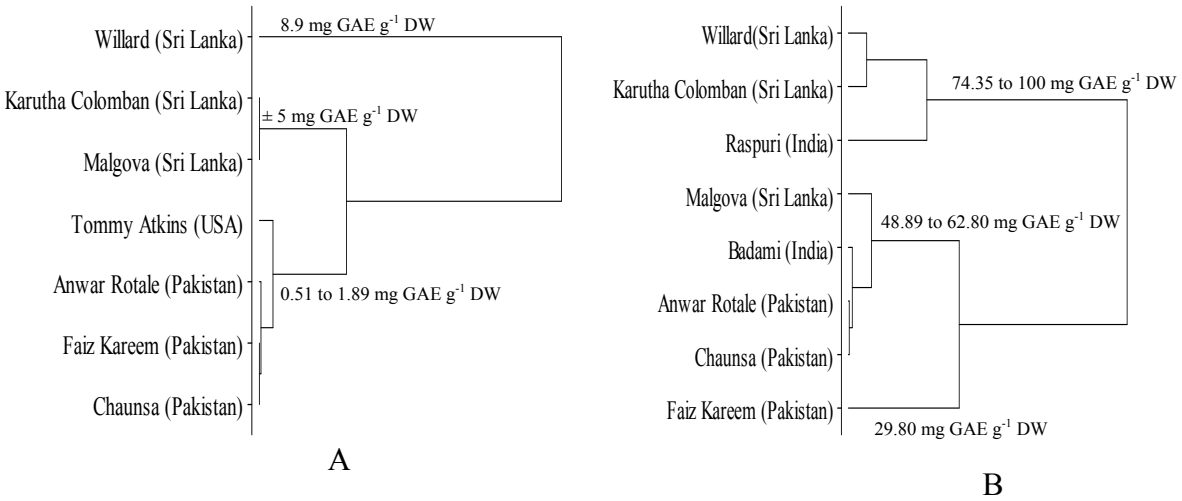


Figure 7.8. Hierarchical cluster analysis dendrogram of TP. Clustering of mango cultivars was based on the TP concentration in pulp (A) and peel (B) at the fully ripe stage. The TP

content of cultivars were extracted and analysed using a similar method. The TP data (Appendix C) was incorporated with the findings of this study and PCA was performed in Genstat

7.3.6 Volatiles

Aroma is an important quality factor which influences marketing of fruits. Volatile compounds are responsible for the aroma and overall flavour of mango fruit (Brackmann *et al*., 1993). Ocimene was the major volatile compound in mango cv. Karutha Colomban whilst terpinolene was responsible for the aroma characteristics of mango cv. Willard. Volatile compounds *viz.* α-pinene, 3-carene and β-caryophyllene also contributed considerably to the aroma of cv. Willard. However, myrcene, ocimene, β-caryophyllene, α-pinene and α-humulene were the main volatiles measured in cv. Malgova with a dominance of myrcene. The total measured volatiles was higher in cv. Malgova followed by cvs. Karutha Colomban and Willard. The prominent volatiles found in Sri Lankan mango cultivars were mostly terpenes, therefore the increase in terpene concentration during ripening may improve the consumer acceptance due to enhanced flavour properties but consequently could result in greater losses by attracting more pests, diseases and rodents (Byers, 1995). Terpinolene is reported to have floral, sweet and pine-like aroma properties and is also one of the major chemical compounds found in mango sap (Maqbool and Malik, 2008). Ocimene and myrcene are responsible for the 'green aroma' of mango fruit whilst the 3-carene has a reported ripe mango flavour (Engel and Tressl, 2002; Pino and Mesa, 2006).

Mango peel had higher concentration of volatile compounds than pulp in cultivars tested. This may be due to the presence of high unbound (free) volatile compounds (aldehydes, fatty acids and terpenes) in peel than pulp. Some of the glycosidically bound volatiles are also transported from peel to pulp as the integrity of the cell walls decline

during ripening (Gomez-Lim, 1997). Therefore, mango peel could also be utilised by the pharmaceutical industry to produce, for example, cosmetic products.

In general, Sri Lankan mango pulp showed lower variation in volatile compounds than peel during ripening at different temperatures. Volatile content increased during lower temperature ripening than higher temperature ripening in cv. Willard pulp whilst decreased in peel. However, the concentration of principle volatile compounds increased in cv. Karutha Colomban fruit pulp during higher temperature ripening. Temperature shock during ripening generally decreased volatile content. Ripening temperature is one of the important factors that influence volatile concentration since it enhances lipid metabolism. Volatile acids, esters and lactones are usually developed by lipid metabolism during fruit ripening. Reduction of volatile concentration during lower temperature ripening may be due to the inhibition of enzymes involved in the metabolic pathway and suppression of ethylene biosynthesis (Singh *et al*., 2004). Ripening temperature influences on enzyme activity in fruits. Mild temperature (20-25^{o}C) increases enzyme activity, yet activities may be reduced at 30^{o}C and is inactivated at higher temperature.

The type and concentration of volatile compounds responsible for the characteristic aroma of mango fruit varied significantly among Sri Lankan mango cultivars tested. The levels and composition of volatile compounds in mango fruit depend on various factors i.e genotypes, pre-harvest factors (Shibamoto and Tang, 1990), harvest maturity and harvesting (Lalel *et al*., 2003a), handling, metabolic pathway during ripening (Quijano *et al*., 2007), postharvest storage conditions (temperature, gas composition, etc.) (Lalel *et al*., 2005), postharvest treatments and processing (Lalel *et al*., 2003b; Quijano *et al*., 2007) and chilling injury (Nair *et al*., 2003). The overall concentration of volatiles was higher in Sri Lankan cultivars than commercial cultivars such as Kensington Pride and Delta R2E2 (Appendix C). However, extraction and quantification method may influence on the final concentration of

volatiles. Therefore, Sri Lankan mango cultivars may attain higher consumer acceptance in global market than commercial mango cultivars.

7.4 Conclusions

Fully mature Sri Lankan mango fruit contained higher starch and lower organic acids than half-mature and immature mango fruit. This maturity stage was confirmed as the optimum harvest maturity since it would enhance biochemical composition and fruit quality during the subsequent postharvest ripening. Ripening temperature is an important factor that influences the concentration of biochemical constituents and therefore the eating quality of the mango fruit. In general, sugar/acid ratio and total carotenoids of mango fruit are improved at higher temperature ripening. A trade off exists, however, since shelf-life will be restricted (*viz*. TP, flavonoids and ascorbic acid) were better maintained during lower temperature ripening. Therefore, lower temperature (20^{o}C) ripening should be encouraged over higher temperature ripening.

Importantly and overall, the Sri Lankan mango cultivars tested in this study (Willard and Karutha Colomban) contained comparatively higher concentration of quality-related compounds compared to most commercial cultivars, but were comparable with some of the Indian and Pakistani mango cultivars. Therefore, cvs. Willard and Karutha Colomban may attain better consumer acceptance not only domestically, but may also represent an opportunity for export.

7.5 Recommendations and Suggestions for further work

Biochemical profiles of five prominent mango cultivars endemic to Sri Lanka were analysed at their pre-climacteric stages in order to understand the optimum harvest maturity, but only three cultivars were selected for the subsequent postharvest studies according to the

biochemical profiles. Furthermore, cvs. Willard and Karutha Colomban could only be suggested for inclusion on the export market based on the significance in nutritional composition and quality parameters. Therefore, it is necessity to carry out more scientific investigation on genotypic variation of cultivars endemic to Sri Lanka to evaluate acceptable qualities to potentially attract overseas market.

Mango fruits are generally harvested at fully mature stage (130 – 140 DAFB) to produce high quality fruits whilst fruits are sometimes harvested at the onset of harvest maturity to extend the ripening period for domestic transport. However, this practice is not encouraged as it yields poor quality in terms of aroma and colour, and also lower concentration of biochemical compounds than fully mature mangoes. Harvest maturity of mango fruit is established based on either of shape, size, colour, DAFB and nutritional composition or combination of two or more of them. It is advisable to consider two or more parameters with DAFB when selecting mango fruits for export market because it is important to maintain the quality and shelf-life to attain the market demand. Generally, mango export takes three to four days to reach the selling or storage point from the harvest, therefore if mango fruits were harvested at fully mature stage and transported at ambient temperature, the shelf-life may be short to maintain the quality for higher consumer acceptance. Therefore, it can be recommend that fully mature mango fruits need to be transported in lower temperature to extend the shelf-life while maintaining quality and nutritional composition. There is no published information on Sri Lankan mango fruits on testing different harvest maturities in relation to the change in quality, shelf-life and biochemical compounds or storage (transportation) studies at lower temperatures. Therefore, further studies are in need to determine the appropriate harvest maturity and export conditions for maintaining the better quality and shelf-life of exported Sri Lankan mango fruits.

Phytohormones and endogenous polyamines play a major role in fruit drop in mango. Exogenous application of various plant growth regulators (gibberellic acid and polyamines) reduces fruit drop (Mengel and Kirby, 1987; Bains *et al*., 1999). Flower and fruit drop is one of the major causes of higher pre- and postharvest losses of mango fruit in Sri Lanka. Deficiency of growth regulators induces the detachment of pod from the stem. However, there is a little published information in Sri Lanka regarding the management of growth regulators to reduce flower and fruit loss. Therefore, further research is needed in Sri Lanka to reduce flower and fruit drops through the management of endogenous plant growth regulators and exogenous application.

Fresh mango fruit pulp has usually been consumed widely in the tropics as a dessert for mainly its flavour and taste. Unripe mango pulp has also been eaten fresh and processed for pickles. However, mango peel is also rich in total phenolics, flavonoids, ascorbic acid and carotenoids, but has not really been utilised to any great extent. Therefore, more research is required to explore ways of better utilising mango peel and possible methods of incorporating these bioactives into the human dietary system.

At present, postharvest storage and packaging facilities of mango fruit are poor in Sri Lanka, therefore techniques like edible coatings, irradiation treatment and control atmosphere storage need to be improved to enhance long-term transportation. Alternative processing methods are necessary for fruits that do not meet the required standards for the fresh market. Additionally, more research is also required to investigate closely the effects of external factors during storage and ripening

Ethylene production plays a significant role in the quality of mango fruit during ripening yet this has not been reported for Sri Lankan mango fruits. It is therefore necessary to be study the impact, control and indeed use of ethylene in detail and optimise ripening conditions.

Softening of mango fruit during ripening results in desirable texture of the fruit pulp, but this in-turn reduces shelf-life due to greater propensity for damage. Textural softening is primarily due to cell wall modification resulting from the structural changes in starch and non-starch polysaccharides. Changes in fruit firmness and structural and non-structural carbohydrates of mango fruits during ripening have been studied globally, but there is no published information on Sri Lankan mangoes. Therefore, firmness and structural carbohydrates of Sri Lankan mango fruits needs to be studied extensively.

Incidence of pest and disease is one of the major problems faced in the mango industry. Sap burn injury and subsequent stem-end rot causes significant problems for producers and exporters since these issues result in poorer quality of mango fruits after harvest (Maqbool and Malik, 2008). Measures are being carried out in several mango producing countries to extend shelf-life of fruit by reducing sap burn injuries, however no research work has been reported on Sri Lankan mangoes. Anthracnose is another important pest which causes severe economic losses to the mango industry and spreads extensively during subsequent postharvest ripening. Fungicide application is reported to be as effective in controlling anthracnose infestation on pre-climacteric mangoes, but there are no appropriate fungicide-free control measures for anthracnose control for postharvest mango fruits. The fungicide application during the postharvest life of fruits is commonly discouraged; therefore extensive research is needed in biological and/or integrated pest management control measures.

Sri Lankan mango fruits are comparatively more fibrous than commercial mango cultivars, fibre needs to be added in our daily diet to aid in digestion and constipation. Therefore studies are needed on extraction and quantification of dietary fibre, which has not been carried out in this study. Individual polyphenolic compounds and carotenoids of mango cultivars endemic to Sri Lanka were also not quantified in this study, therefore further

studies are needed to extract and quantify said compounds. Sri Lankan mango fruits are rich in antioxidants and may have higher antioxidant capacity than commercial cultivars.

Sensory evaluation using taste panels needs to be done to verify the changes in sugar/acid ratio and aroma that affects taste preference. Market study and consumer satisfaction surveys may also be vital to understand consumer acceptability of Sri Lankan mango fruits based on their taste, aroma, colour, texture, dietary fibre and health-promoting properties. This work would add further confidence to the export possibilities of Sri Lankan mango fruits. In general, consumers purchase mango fruits in Sri Lankan domestic market based on cultivars. Local consumers usually prefer fully ripen mango fruits of cvs. Willard and Karutha Colomban for immediate consumption since they posses better taste, colour and aroma than other Sri Lankan mango fruits whilst consumers purchase mango fruit at fully mature or half-ripen stages for delayed consumption, domestic transportation and wholesale. However, there is no reported information on consumer studies or taste panel recommendations for taste, aroma and colour of Sri Lankan mango fruits. Therefore, consumer study information would have been more supportive to the findings of aroma compounds of this study to optimise the handling practices and sale. Volatile composition is specific to cultivars, for example terpinolene is abundant in cv. Willard whilst ocimene and myrcene are prominent in cvs. Karutha Colomban and Malgove, respectively. Each volatile compound has its own characteristic features, since consumers prefer cv. Willard fruit than other Sri Lankan mango fruits (terpinolene has floral and sweet aroma), it is assumed that terpinolene is a highly acceptable aroma by many consumers. However, this observation might have been validated by a consumer study.

Harvest maturity, harvesting techniques and postharvest operations such as storage, transportation, ripening and packaging are the general handling practices that affect biochemical composition of mango fruit and quality parameters such as volatile content,

colour, taste and texture. Therefore, it is necessary to conduct extensive consumer studies and taste panels on taste, colour, aroma and appearance in order to optimise handling practices to produce quality mango fruits.

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APPENDICES

APPENDIX A: STATISTICAL TABLES

A.3.1: Analysis of variance (ANOVA) of non-structural carbohydrates of pre-climacteric mango analysis

A.3.1.1 ANOVA of total measured sugars (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars_C1_C5	4	1396519.	349130.	80.69	<.001
HT	2	628441.	314220.	72.62	<.001
Maturity	2	126311.	63155.	14.60	<.001
VT	2	1319.	659.	0.15	0.859
Cultivars_C1_C5.HT	8	119557.	14945.	3.45	<.001
Cultivars_C1_C5.Maturity	8	435149.	54394.	12.57	<.001
HT.Maturity	4	37469.	9367.	2.16	0.073
Cultivars_C1_C5.VT	8	35823.	4478.	1.03	0.410
HT.VT	4	14756.	3689.	0.85	0.493
Maturity.VT	4	4435.	1109.	0.26	0.906
Cultivars_C1_C5.HT.Maturity	16	98645.	6165.	1.42	0.129
Cultivars_C1_C5.HT.VT	16	19984.	1249.	0.29	0.997
Cultivars_C1_C5.Maturity.VT	16	33523.	2095.	0.48	0.954
HT.Maturity.VT	8	11360.	1420.	0.33	0.955
Cultivars_C1_C5.HT.Maturity.VT	32	49743.	1554.	0.36	1.000
Residual	270	1168293.	4327.		
Total	404	4181326.			

A.3.1.2: ANOVA of fructose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars_C1_C5	4	247948.	61987.	58.39	<.001
HT	2	69942.	34971.	32.94	<.001
Maturity	2	3528.	1764.	1.66	0.192
VT	2	1467.	733.	0.69	0.502
Cultivars_C1_C5.HT	8	17910.	2239.	2.11	0.035
Cultivars_C1_C5.Maturity	8	130119.	16265.	15.32	<.001
HT.Maturity	4	4135.	1034.	0.97	0.422
Cultivars_C1_C5.VT	8	6824.	853.	0.80	0.600
HT.VT	4	5306.	1326.	1.25	0.290
Maturity.VT	4	1930.	482.	0.45	0.769
Cultivars_C1_C5.HT.Maturity	16	17758.	1110.	1.05	0.409
Cultivars_C1_C5.HT.VT	16	4711.	294.	0.28	0.998
Cultivars_C1_C5.Maturity.VT	16	12107.	757.	0.71	0.780
HT.Maturity.VT	8	4481.	560.	0.53	0.835
Cultivars_C1_C5.HT.Maturity.VT	32	10758.	336.	0.32	1.000
Residual	270	286625.	1062.		
Total	404	825547.			

A.3.1.3: ANOVA of glucose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars_C1_C5	4	269039.5	67259.9	75.55	<.001
HT	2	55021.5	27510.7	30.90	<.001
Maturity	2	84062.1	42031.1	47.21	<.001
VT	2	53.1	26.5	0.03	0.971
Cultivars_C1_C5.HT	8	39265.2	4908.1	5.51	<.001
Cultivars_C1_C5.Maturity	8	133409.5	16676.2	18.73	<.001
HT.Maturity	4	25310.2	6327.6	7.11	<.001
Cultivars_C1_C5.VT	8	7786.7	973.3	1.09	0.368
HT.VT	4	2903.0	725.7	0.82	0.516
Maturity.VT	4	640.4	160.1	0.18	0.949
Cultivars_C1_C5.HT.Maturity	16	50883.4	3180.2	3.57	<.001
Cultivars_C1_C5.HT.VT	16	3777.6	236.1	0.27	0.998
Cultivars_C1_C5.Maturity.VT	16	5451.0	340.7	0.38	0.986
HT.Maturity.VT	8	1821.7	227.7	0.26	0.979
Cultivars_C1_C5.HT.Maturity.VT	32	9128.5	285.3	0.32	1.000
Residual	270	240379.1	890.3		
Total	404	928932.5			

A.3.1.4: ANOVA of sucrose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars_C1_C5	4	61640.4	15410.1	51.80	<.001
HT	2	96211.7	48105.8	161.69	<.001
Maturity	2	7673.8	3836.9	12.90	<.001
VT	2	93.4	46.7	0.16	0.855
Cultivars_C1_C5.HT	8	9570.9	1196.4	4.02	<.001
Cultivars_C1_C5.Maturity	8	44195.2	5524.4	18.57	<.001
HT.Maturity	4	4638.6	1159.7	3.90	0.004
Cultivars_C1_C5.VT	8	2230.4	278.8	0.94	0.486
HT.VT	4	257.2	64.3	0.22	0.929
Maturity.VT	4	572.9	143.2	0.48	0.749
Cultivars_C1_C5.HT.Maturity	16	25523.0	1595.2	5.36	<.001
Cultivars_C1_C5.HT.VT	16	2501.3	156.3	0.53	0.933
Cultivars_C1_C5.Maturity.VT	16	1191.2	74.4	0.25	0.999
HT.Maturity.VT	8	1407.4	175.9	0.59	0.785
Cultivars_C1_C5.HT.Maturity.VT	32	4166.1	130.2	0.44	0.997
Residual	270	80330.0	297.5		
Total	404	342203.4			

A.3.1.5: ANOVA of starch (% DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	8028.70	4014.35	46.68	<.001
HT	2	2124.00	1062.00	12.35	<.001
Maturity	2	14835.63	7417.82	86.25	<.001
VT	2	41.43	20.72	0.24	0.786
Cultivars.HT	4	66.18	16.54	0.19	0.942
Cultivars.Maturity	4	12873.52	3218.38	37.42	<.001
HT.Maturity	4	871.61	217.90	2.53	0.042
Cultivars.VT	4	100.61	25.15	0.29	0.883
HT.VT	4	164.32	41.08	0.48	0.752
Maturity.VT	4	378.09	94.52	1.10	0.359
Cultivars.HT.Maturity	8	372.26	46.53	0.54	0.824
Cultivars.HT.VT	8	131.20	16.40	0.19	0.992
Cultivars.Maturity.VT	8	214.06	26.76	0.31	0.961
HT.Maturity.VT	8	63.58	7.95	0.09	0.999
Cultivars.HT.Maturity.VT	16	324.11	20.26	0.24	0.999
Residual	162	13931.80	86.00		
Total	242	54521.11			

APPENDIX A.3.2: Analysis of variance (ANOVA) of non-volatile organic acids of pre-climacteric mango analysis

A.3.2.1: ANOVA of citric acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars_C1_C5	4	250561.5	62640.4	75.56	<.001
HT	2	1514317.7	757158.9	913.27	<.001
Maturity	2	159117.1	79558.5	95.96	<.001
VT	2	947.0	473.5	0.57	0.566
Cultivars_C1_C5.HT	8	125154.8	15644.4	18.87	<.001
Cultivars_C1_C5.Maturity	8	305385.1	38173.1	46.04	<.001
HT.Maturity	4	73214.5	18303.6	22.08	<.001
Cultivars_C1_C5.VT	8	17459.4	2182.4	2.63	0.009
HT.VT	4	629.1	157.3	0.19	0.944
Maturity.VT	4	11379.8	2844.9	3.43	0.009
Cultivars_C1_C5.HT.Maturity	16	183097.4	11443.6	13.80	<.001
Cultivars_C1_C5.HT.VT	16	26763.6	1672.7	2.02	0.012
Cultivars_C1_C5.Maturity.VT	16	63722.1	3982.6	4.80	<.001
HT.Maturity.VT	8	4073.8	509.2	0.61	0.766
Cultivars_C1_C5.HT.Maturity.VT	32	57211.0	1787.8	2.16	<.001
Residual	270	223847.9	829.1		
Total	404	3016881.7			

A.3.2.2: ANOVA of malic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars_C1_C5	4	2575.4	643.8	2.75	0.029
HT	2	100.1	50.1	0.21	0.808
Maturity	2	3991.0	1995.5	8.52	<.001
VT	2	1245.2	622.6	2.66	0.072
Cultivars_C1_C5.HT	8	486.8	60.9	0.26	0.978
Cultivars_C1_C5.Maturity	8	5570.9	696.4	2.97	0.003
HT.Maturity	4	57.8	14.4	0.06	0.993
Cultivars_C1_C5.VT	8	3083.5	385.4	1.65	0.112
HT.VT	4	146.4	36.6	0.16	0.960
Maturity.VT	4	2622.5	655.6	2.80	0.026
Cultivars_C1_C5.HT.Maturity	16	641.0	40.1	0.17	1.000
Cultivars_C1_C5.HT.VT	16	579.3	36.2	0.15	1.000
Cultivars_C1_C5.Maturity.VT	16	6932.2	433.3	1.85	0.025
HT.Maturity.VT	8	198.5	24.8	0.11	0.999
Cultivars_C1_C5.HT.Maturity.VT	32	1096.2	34.3	0.15	1.000
Residual	270	63201.9	234.1		
Total	404	92528.8			

A.3.2.3: ANOVA of oxalic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars_C1_C5	4	69.875	17.469	14.97	<.001
HT	2	4.515	2.258	1.93	0.146
Maturity	2	14.465	7.232	6.20	0.002
VT	2	2.394	1.197	1.03	0.360
Cultivars_C1_C5.HT	8	5.513	0.689	0.59	0.786
Cultivars_C1_C5.Maturity	8	47.279	5.910	5.06	<.001
HT.Maturity	4	2.711	0.678	0.58	0.677
Cultivars_C1_C5.VT	8	16.537	2.067	1.77	0.083
HT.VT	4	4.417	1.104	0.95	0.438
Maturity.VT	4	15.593	3.898	3.34	0.011
Cultivars_C1_C5.HT.Maturity	16	12.432	0.777	0.67	0.827
Cultivars_C1_C5.HT.VT	16	13.970	0.873	0.75	0.743
Cultivars_C1_C5.Maturity.VT	16	42.320	2.645	2.27	0.004
HT.Maturity.VT	8	8.847	1.106	0.95	0.478
Cultivars_C1_C5.HT.Maturity.VT	32	36.593	1.144	0.98	0.503
Residual	270	315.086	1.167		
Total	404	612.547			

A.3.2.4: ANOVA of tartaric acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars_C1_C5	4	117.838	29.459	5.42	<.001
HT	2	159.732	79.866	14.70	<.001
Maturity	2	189.906	94.953	17.48	<.001
VT	2	126.577	63.289	11.65	<.001
Cultivars_C1_C5.HT	8	111.728	13.966	2.57	0.010
Cultivars_C1_C5.Maturity	8	659.424	82.428	15.17	<.001
HT.Maturity	4	156.273	39.068	7.19	<.001
Cultivars_C1_C5.VT	8	531.339	66.417	12.23	<.001
HT.VT	4	46.712	11.678	2.15	0.075
Maturity.VT	4	68.883	17.221	3.17	0.014
Cultivars_C1_C5.HT.Maturity	16	249.744	15.609	2.87	<.001
Cultivars_C1_C5.HT.VT	16	235.155	14.697	2.71	<.001
Cultivars_C1_C5.Maturity.VT	16	799.971	49.998	9.20	<.001
HT.Maturity.VT	8	18.847	2.356	0.43	0.900
Cultivars_C1_C5.HT.Maturity.VT	32	580.598	18.144	3.34	<.001
Residual	270	1466.887	5.433		
Total	404	5519.614			

A.3.2.5: ANOVA of total measured organic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars_C1_C5	4	202801.	50700.	42.49	<.001
HT	2	1525243.	762622.	639.12	<.001
Maturity	2	219319.	109660.	91.90	<.001
VT	2	3419.	1709.	1.43	0.241
Cultivars_C1_C5.HT	8	139022.	17378.	14.56	<.001
Cultivars_C1_C5.Maturity	8	332699.	41587.	34.85	<.001
HT.Maturity	4	76292.	19073.	15.98	<.001
Cultivars_C1_C5.VT	8	25934.	3242.	2.72	0.007
HT.VT	4	1118.	280.	0.23	0.919
Maturity.VT	4	11266.	2817.	2.36	0.054
Cultivars_C1_C5.HT.Maturity	16	183594.	11475.	9.62	<.001
Cultivars_C1_C5.HT.VT	16	25688.	1605.	1.35	0.169
Cultivars_C1_C5.Maturity.VT	16	77666.	4854.	4.07	<.001
HT.Maturity.VT	8	5486.	686.	0.57	0.798
Cultivars_C1_C5.HT.Maturity.VT	32	58000.	1813.	1.52	0.041
Residual	270	322175.	1193.		
Total	404	3209722.			

APPENDIX A.3.3: Analysis of variance (ANOVA) of total phenolics of pre-climacteric mango analysis (mg GAE g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars_C1_C5	4	1955.1	488.8	2.32	0.057
HT	2	543.3	271.6	1.29	0.277
Maturity	2	1008.9	504.5	2.39	0.093
VT	2	81.6	40.8	0.19	0.824
Cultivars_C1_C5.HT	8	486.8	60.9	0.29	0.969
Cultivars_C1_C5.Maturity	8	1391.1	173.9	0.83	0.581
HT.Maturity	4	117.4	29.3	0.14	0.968
Cultivars_C1_C5.VT	8	1421.3	177.7	0.84	0.565
HT.VT	4	37.5	9.4	0.04	0.996
Maturity.VT	4	424.3	106.1	0.50	0.733
Cultivars_C1_C5.HT.Maturity	16	158.3	9.9	0.05	1.000
Cultivars_C1_C5.HT.VT	16	168.4	10.5	0.05	1.000
Cultivars_C1_C5.Maturity.VT	16	3119.9	195.0	0.93	0.540
HT.Maturity.VT	8	172.3	21.5	0.10	0.999
Cultivars_C1_C5.HT.Maturity.VT	32	517.6	16.2	0.08	1.000
Residual	270	56886.0	210.7		
Total	404	68489.7			

APPENDIX A.4.1: Analysis of variance (ANOVA) of sugars of postharvest mango ripened at 32^{o}C

A.4.1.1: ANOVA of total measured sugars (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	21231.	10615.	5.38	0.009
Storage	2	16164.	8082.	4.09	0.025
sections	1	1687070.	1687070.	854.32	<.001
Cultivars.Storage	4	24208.	6052.	3.06	0.029
Cultivars.sections	2	12916.	6458.	3.27	0.050
Storage.sections	2	9118.	4559.	2.31	0.114
Cultivars.Storage.sections	4	1526.	382.	0.19	0.940
Residual	36	71091.	1975.		
Total	53	1843324.			

A.4.1.2: ANOVA of fructose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	6931.9	3466.0	6.61	0.004
Storage	2	6675.0	3337.5	6.36	0.004
sections	1	11442.6	11442.6	21.81	<.001
Cultivars.Storage	4	5077.0	1269.2	2.42	0.066
Cultivars.sections	2	13130.4	6565.2	12.51	<.001
Storage.sections	2	5012.3	2506.1	4.78	0.014
Cultivars.Storage.sections	4	2451.9	613.0	1.17	0.341
Residual	36	18887.2	524.6		
Total	53	69608.2			

A.4.1.3: ANOVA of glucose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	11634.3	5817.1	48.87	<.001
Storage	2	4381.2	2190.6	18.40	<.001
sections	1	77.7	77.7	0.65	0.424
Cultivars.Storage	4	269.4	67.4	0.57	0.689
Cultivars.sections	2	2506.8	1253.4	10.53	<.001
Storage.sections	2	438.9	219.5	1.84	0.173
Cultivars.Storage.sections	4	629.4	157.4	1.32	0.280
Residual	36	4285.0	119.0		
Total	53	24222.8			

A.4.1.4: ANOVA of sucrose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	43942.	21971.	8.71	<.001
Storage	2	29408.	14704.	5.83	0.006
sections	1	1399693.	1399693.	554.76	<.001
Cultivars.Storage	4	33059.	8265.	3.28	0.022
Cultivars.sections	2	44312.	22156.	8.78	<.001
Storage.sections	2	39.	20.	0.01	0.992
Cultivars.Storage.sections	4	1699.	425.	0.17	0.953
Residual	36	90829.	2523.		
Total	53	1642982.			

APPENDIX A.4.2: Analysis of variance (ANOVA) of non-volatile organic acids of postharvest mango ripened at 32^{o}C

A.4.2.1: ANOVA of ascorbic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	484.0149	242.0075	538.35	<.001
Storage	2	2.9027	1.4513	3.23	0.051
sections	1	7.7278	7.7278	17.19	<.001
Cultivars.Storage	4	2.0238	0.5060	1.13	0.360
Cultivars.sections	2	19.9782	9.9891	22.22	<.001
Storage.sections	2	0.2962	0.1481	0.33	0.721
Cultivars.Storage.sections	4	0.6046	0.1511	0.34	0.852
Residual	36	16.1832	0.4495		
Total	53	533.7313			

A.4.2.2: ANOVA of citric acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	1951.80	975.90	16.51	<.001
Storage	2	6029.72	3014.86	51.00	<.001
sections	1	4232.94	4232.94	71.61	<.001
Cultivars.Storage	4	1622.88	405.72	6.86	<.001
Cultivars.sections	2	1909.04	954.52	16.15	<.001
Storage.sections	2	2937.11	1468.55	24.84	<.001
Cultivars.Storage.sections	4	1708.71	427.18	7.23	<.001
Residual	36	2127.99	59.11		
Total	53	22520.19			

A.4.2.3: ANOVA of malic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	27.503	13.752	2.06	0.142
Storage	2	5.549	2.774	0.42	0.663
sections	1	119.337	119.337	17.86	<.001
Cultivars.Storage	4	12.905	3.226	0.48	0.748
Cultivars.sections	2	8.095	4.047	0.61	0.551
Storage.sections	2	2.217	1.108	0.17	0.848
Cultivars.Storage.sections	4	7.500	1.875	0.28	0.889
Residual	36	240.537	6.682		
Total	53	423.642			

A.4.2.4: ANOVA of oxalic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	8.847	4.424	4.42	0.019
Storage	2	10.001	5.000	5.00	0.012
sections	1	0.285	0.285	0.29	0.597
Cultivars.Storage	4	2.778	0.695	0.69	0.601
Cultivars.sections	2	2.713	1.356	1.36	0.271
Storage.sections	2	1.190	0.595	0.59	0.557
Cultivars.Storage.sections	4	7.480	1.870	1.87	0.137
Residual	36	36.028	1.001		
Total	53	69.323			

A.4.2.5: ANOVA of tartaric acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	62.7364	31.3682	42.78	<.001
Storage	2	0.7824	0.3912	0.53	0.591
sections	1	30.2602	30.2602	41.27	<.001
Cultivars.Storage	4	8.9343	2.2336	3.05	0.029
Cultivars.sections	2	6.1356	3.0678	4.18	0.023
Storage.sections	2	0.5790	0.2895	0.39	0.677
Cultivars.Storage.sections	4	1.9101	0.4775	0.65	0.630
Residual	36	26.3949	0.7332		
Total	53	137.7329			

A.4.2.6: ANOVA of total measured organic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	1856.58	928.29	12.06	<.001
Storage	2	6697.63	3348.82	43.49	<.001
sections	1	2152.06	2152.06	27.95	<.001
Cultivars.Storage	4	1844.86	461.21	5.99	<.001
Cultivars.sections	2	2072.89	1036.44	13.46	<.001
Storage.sections	2	2994.78	1497.39	19.45	<.001
Cultivars.Storage.sections	4	1973.90	493.47	6.41	<.001
Residual	36	2772.05	77.00		
Total	53	22364.75			

APPENDIX A.4.3: Analysis of variance (ANOVA) of total phenolics of postharvest mango ripened at 32^{o}C (mg GAE g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	4055.0	2027.5	11.18	<.001
Storage	2	877.0	438.5	2.42	0.103
sections	1	41645.8	41645.8	229.60	<.001
Cultivars.Storage	4	874.6	218.6	1.21	0.325
Cultivars.sections	2	3253.1	1626.6	8.97	<.001
Storage.sections	2	713.1	356.6	1.97	0.155
Cultivars.Storage.sections	4	872.3	218.1	1.20	0.327
Residual	36	6530.0	181.4		
Total	53	58820.9			

APPENDIX A.4.4: Analysis of variance (ANOVA) of flavonoids of postharvest mango ripened at 32^{o}C (µg g^{-1} DW)

A.4.4.1: ANOVA of mangiferin (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	39516888.	19758444.	9.38	0.002
Storage	2	6031782.	3015891.	1.43	0.265
Cultivars.Storage	4	47506127.	11876532.	5.64	0.004
Residual	18	37925910.	2106995.		
Total	26	130980707.			

A.4.4.2: ANOVA of quercetin O 3 galactoside (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	487825.	243912.	56.25	<.001
Storage	2	13394.	6697.	1.54	0.240
Cultivars.Storage	4	16028.	4007.	0.92	0.472
Residual	18	78047.	4336.		
Total	26	595294.			

A.4.4.3: ANOVA of quercetin O 3 glucoside (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	255460.	127730.	20.00	<.001
Storage	2	28287.	14143.	2.21	0.138
Cultivars.Storage	4	24727.	6182.	0.97	0.449
Residual	18	114944.	6386.		
Total	26	423418.			

A.4.4.4: ANOVA of quercetin O 3 rhamnoside (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	226.0	113.0	0.25	0.784
Storage	2	582.9	291.4	0.63	0.542
Cultivars.Storage	4	4374.3	1093.6	2.38	0.090
Residual	18	8268.2	459.3		
Total	26	13451.3			

A.4.4.5: ANOVA of kaemferol O 3 glucoside (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	6407.	3204.	1.36	0.281
Storage	2	4827.	2413.	1.03	0.378
Cultivars.Storage	4	2420.	605.	0.26	0.901
Residual	18	42265.	2348.		
Total	26	55919.			

A.4.4.6: ANOVA of quercetin (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	1363.6	681.8	5.61	0.013
Storage	2	569.6	284.8	2.34	0.125
Cultivars.Storage	4	1696.1	424.0	3.49	0.028
Residual	18	2189.0	121.6		
Total	26	5818.4			

APPENDIX A.4.5: Analysis of variance (ANOVA) of total carotenoids of postharvest mango ripened at 32^{o}C (µg g^{-1} FW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	8742.48	4371.24	46.97	<.001
Storage	2	7205.72	3602.86	38.71	<.001
Cultivars.Storage	4	696.67	174.17	1.87	0.159
Residual	18	1675.22	93.07		
Total	26	18320.09			

APPENDIX A.4.6: Analysis of variance (ANOVA) of total titratable acidity (TTA) of postharvest mango ripened at 32^{o}C (mg g^{-1} FW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
cultivars	2	30.77106	15.38553	200.89	<.001
storage	2	204.27266	102.13633	1333.62	<.001
cultivars.storage	4	14.15826	3.53957	46.22	<.001
Residual	18	1.37854	0.07659		
Total	26	250.58053			

APPENDIX A.4.7: Analysis of variance (ANOVA) of total soluble solids (TSS) of postharvest mango ripened at 32°C (% DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
cultivars	2	84.429	42.214	7.78	0.004
storage	2	59.876	29.938	5.52	0.014
cultivars.storage	4	25.022	6.256	1.15	0.364
Residual	18	97.620	5.423		
Total	26	266.947			

APPENDIX A.4.8: Analysis of variance (ANOVA) of sugars/acid ratio of postharvest mango ripened at 32°C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
cultivars	2	7354.4	3677.2	13.12	<.001
storage	2	15809.9	7904.9	28.21	<.001
cultivars.storage	4	5682.2	1420.5	5.07	0.006
Residual	18	5043.5	280.2		
Total	26	33889.9			

APPENDIX A.4.9: Analysis of variance (ANOVA) of TSS/acid ratio of postharvest mango ripened at 32°C

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
cultivars	2	7354.4	3677.2	13.12	<.001
storage	2	15809.9	7904.9	28.21	<.001
cultivars.storage	4	5682.2	1420.5	5.07	0.006
Residual	18	5043.5	280.2		
Total	26	33889.9			

APPENDIX A.5.1a: Analysis of variance (ANOVA) of sugars of postharvest mango cv. Willard ripened at 30°C and 20°C with temperature variations

A.5.1a.1: ANOVA of total measured sugars (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	4	30223.	7556.	1.99	0.124
Out_turn	2	11953.	5977.	1.57	0.225
Tissue	1	978176.	978176.	257.52	<.001
CT_tresatment.Tissue	4	23891.	5973.	1.57	0.209
Out_turn.Tissue	2	18565.	9282.	2.44	0.105
Residual	28	106356.	3798.		
Total	41	1169164.			

A.5.1a.2: ANOVA of fructose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	4	6977.	1744.	1.07	0.389
Out_turn	2	2741.	1371.	0.84	0.441
Tissue	1	33385.	33385.	20.51	<.001
CT_tresatment.Tissue	4	4273.	1068.	0.66	0.627
Out_turn.Tissue	2	3102.	1551.	0.95	0.398
Residual	28	45580.	1628.		
Total	41	96058.			

A.5.1a.3: ANOVA of glucose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	4	17405.8	4351.5	24.70	<.001
Out_turn	2	7760.5	3880.2	22.03	<.001
Tissue	1	4818.8	4818.8	27.36	<.001
CT_tresatment.Tissue	4	281.0	70.3	0.40	0.808
Out_turn.Tissue	2	491.4	245.7	1.39	0.265
Residual	28	4932.4	176.2		
Total	41	35689.8			

A.5.1a.4: ANOVA of sucrose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	4	77524.	19381.	8.48	<.001
Out_turn	2	21045.	10522.	4.60	0.019
Tissue	1	543016.	543016.	237.52	<.001
CT_tresatment.Tissue	4	17806.	4451.	1.95	0.130
Out_turn.Tissue	2	10552.	5276.	2.31	0.118
Residual	28	64014.	2286.		
Total	41	733957.			

APPENDIX A.5.1b: Analysis of variance (ANOVA) of sugars of postharvest mango cv. Karutha Colomban ripened at 30^{o}C and 20^{o}C with temperature variations

A.5.1b.1: ANOVA of total measured sugars (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	3	19699.	6566.	1.65	0.213
Out_turn	2	14575.	7288.	1.83	0.189
Tissue	1	1330309.	1330309.	334.10	<.001
CT_tresatment.Tissue	3	19567.	6522.	1.64	0.216
Out_turn.Tissue	2	230.	115.	0.03	0.972
Residual	18	71671.	3982.		
Total	29	1456051.			

A.5.1b.2: ANOVA of fructose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	3	2810.	937.	0.59	0.632
Out_turn	2	7773.	3886.	2.43	0.116
Tissue	1	25099.	25099.	15.71	<.001
CT_tresatment.Tissue	3	2016.	672.	0.42	0.740
Out_turn.Tissue	2	1404.	702.	0.44	0.651
Residual	18	28752.	1597.		
Total	29	67854.			

A.5.1b.3: ANOVA of glucose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	3	4211.1	1403.7	7.59	0.002
Out_turn	2	3024.4	1512.2	8.17	0.003
Tissue	1	352.3	352.3	1.90	0.184
CT_tresatment.Tissue	3	258.1	86.0	0.47	0.710
Out_turn.Tissue	2	2.9	1.5	0.01	0.992
Residual	18	3329.6	185.0		
Total	29	11178.4			

A.5.1b.4: ANOVA of sucrose (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	3	13573.	4524.	1.81	0.182
Out_turn	2	503.	252.	0.10	0.905
Tissue	1	952955.	952955.	381.15	<.001
CT_tresatment.Tissue	3	15722.	5241.	2.10	0.137
Out_turn.Tissue	2	577.	289.	0.12	0.892
Residual	18	45004.	2500.		
Total	29	1028334.			

APPENDIX A.5.2a: Analysis of variance (ANOVA) of organic acids of postharvest mango cv. Willard ripened at 30°C and 20°C with temperature variations

A.5.2a.1: ANOVA of AsA (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	4	74.587	18.647	6.41	<.001
Out_turn	2	32.914	16.457	5.66	0.009
Tissue	1	92.065	92.065	31.67	<.001
CT_tresatment.Tissue	4	6.155	1.539	0.53	0.715
Out_turn.Tissue	2	30.807	15.403	5.30	0.011
Residual	28	81.394	2.907		
Total	41	317.922			

A.5.2a.2: ANOVA of citric acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	4	6652.8	1663.2	12.00	<.001
Out_turn	2	3989.3	1994.6	14.39	<.001
Tissue	1	23410.1	23410.1	168.88	<.001
CT_tresatment.Tissue	4	7716.1	1929.0	13.92	<.001
Out_turn.Tissue	2	3718.3	1859.1	13.41	<.001
Residual	28	3881.3	138.6		
Total	41	49367.8			

A.5.2a.3: ANOVA of malic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	4	44.0	11.0	0.07	0.990
Out_turn	2	289.2	144.6	0.96	0.396
Tissue	1	251.9	251.9	1.67	0.207
CT_tresatment.Tissue	4	653.6	163.4	1.08	0.384
Out_turn.Tissue	2	211.5	105.8	0.70	0.505
Residual	28	4229.8	151.1		
Total	41	5680.0			

A.5.2a.4: ANOVA of oxalic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	4	2.4046	0.6012	5.71	0.002
Out_turn	2	0.7168	0.3584	3.40	0.048
Tissue	1	0.0837	0.0837	0.79	0.380
CT_tresatment.Tissue	4	0.5229	0.1307	1.24	0.317
Out_turn.Tissue	2	0.0101	0.0050	0.05	0.953
Residual	28	2.9504	0.1054		
Total	41	6.6885			

A.5.2a.5: ANOVA of tartaric acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	4	122.04	30.51	1.03	0.411
Out_turn	2	27.01	13.50	0.45	0.640
Tissue	1	730.01	730.01	24.55	<.001
CT_tresatment.Tissue	4	77.94	19.48	0.66	0.628
Out_turn.Tissue	2	1.96	0.98	0.03	0.968
Residual	28	832.73	29.74		
Total	41	1791.69			

A.5.2a.6: ANOVA of total acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	4	8616.9	2154.2	8.07	<.001
Out_turn	2	6651.1	3325.5	12.46	<.001
Tissue	1	42106.3	42106.3	157.76	<.001
CT_tresatment.Tissue	4	6076.4	1519.1	5.69	0.002
Out_turn.Tissue	2	4715.7	2357.8	8.83	0.001
Residual	28	7473.2	266.9		
Total	41	75639.6			

APPENDIX A.5.2b: Analysis of variance (ANOVA) of organic acids of postharvest mango cv. Karutha Colomban ripened at 30^{o}C and 20^{o}C with temperature variations

A.5.2b.1: ANOVA of AsA (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	3	0.8043	0.2681	2.23	0.120
Tissue	1	0.0518	0.0518	0.43	0.520
Out_turn	2	0.1387	0.0694	0.58	0.572
CT_tresatment.Tissue	3	1.9701	0.6567	5.46	0.008
Tissue.Out_turn	2	0.1387	0.0694	0.58	0.572
Residual	18	2.1658	0.1203		
Total	29	5.2694			

A.5.2b.2: ANOVA of citric acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	3	8290.6	2763.5	7.07	0.002
Tissue	1	7412.2	7412.2	18.96	<.001
Out_turn	2	97.3	48.6	0.12	0.884
CT_tresatment.Tissue	3	9509.3	3169.8	8.11	0.001
Tissue.Out_turn	2	265.9	133.0	0.34	0.716
Residual	18	7038.1	391.0		
Total	29	32613.5			

A.5.2b.3: ANOVA of malic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	3	39.30	13.10	0.94	0.444
Tissue	1	189.95	189.95	13.56	0.002
Out_turn	2	2.12	1.06	0.08	0.928
CT_tresatment.Tissue	3	12.33	4.11	0.29	0.829
Tissue.Out_turn	2	0.03	0.01	0.00	0.999
Residual	18	252.08	14.00		
Total	29	495.81			

A.5.2b.4: ANOVA of oxalic acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	3	1.2256	0.4085	1.51	0.245
Tissue	1	0.0015	0.0015	0.01	0.942
Out_turn	2	0.1093	0.0546	0.20	0.819
CT_tresatment.Tissue	3	3.6374	1.2125	4.49	0.016
Tissue.Out_turn	2	0.1238	0.0619	0.23	0.798
Residual	18	4.8647	0.2703		
Total	29	9.9621			

A.5.2b.5: ANOVA of tartaric acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	3	157.45	52.48	1.39	0.279
Tissue	1	31.75	31.75	0.84	0.372
Out_turn	2	0.04	0.02	0.00	0.999
CT_tresatment.Tissue	3	95.55	31.85	0.84	0.489
Tissue.Out_turn	2	0.00	0.00	0.00	1.000
Residual	18	681.99	37.89		
Total	29	966.79			

A.5.2b.6: ANOVA of total acid (mg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_tresatment	3	11104.7	3701.6	6.94	0.003
Tissue	1	4367.3	4367.3	8.19	0.010
Out_turn	2	62.5	31.2	0.06	0.943
CT_tresatment.Tissue	3	7331.5	2443.8	4.58	0.015
Tissue.Out_turn	2	261.7	130.9	0.25	0.785
Residual	18	9595.3	533.1		
Total	29	32723.1			

APPENDIX A.5.3a: Analysis of variance (ANOVA) of TP of postharvest mango cv. Willard ripened at 30°C and 20°C with temperature variations (mg GAE g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT_treatment	6	105.97	17.66	0.41	0.868
Tissue	1	27435.90	27435.90	631.87	<.001
CT_treatment.Tissue	6	124.74	20.79	0.48	0.818
Residual	28	1215.77	43.42		
Total	41	28882.38			

APPENDIX A.5.3b: Analysis of variance (ANOVA) of TP of postharvest mango cv. Karutha Colomban ripened at 30°C and 20°C with temperature variations (mg GAE g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT	4	34.89	8.72	0.13	0.969
Tissue	1	16021.70	16021.70	242.16	<.001
CT.Tissue	4	37.64	9.41	0.14	0.964
Residual	20	1323.25	66.16		
Total	29	17417.49			

APPENDIX A.5.4a: Analysis of variance (ANOVA) of flavonoids of postharvest mango cv. Willard ripened at 30°C and 20°C with temperature variations (µg g^{-1} DW)

A.5.4a.1: ANOVA of mangiferin (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT	4	23416213.	5854053.	0.72	0.596
out_turn	2	1110613.	555307.	0.07	0.935
Residual	13	106234142.	8171857.		
Total	19	130760968.			

A.5.4a.2: ANOVA of quercetin 3 O galactoside (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT	4	89429.	22357.	0.91	0.486
out_turn	2	2073.	1036.	0.04	0.959
Residual	13	318918.	24532.		
Total	19	410420.			

A.5.4a.3: ANOVA of quercetin 3 O glucoside (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT	4	74496.	18624.	1.58	0.238
out_turn	2	2331.	1165.	0.10	0.907
Residual	13	153311.	11793.		
Total	19	230137.			

APPENDIX A.5.4b: Analysis of variance (ANOVA) of flavonoids of postharvest mango cv. Karutha Colomban ripened at 30°C and 20°C with temperature variations (µg g^{-1} DW)

A.5.4b.1: ANOVA of mangiferin (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT	3	3788614.	1262871.	0.20	0.891
out_turn	2	4918318.	2459159.	0.40	0.685
Residual	8	49537720.	6192215.		
Total	13	58244651.			

A.5.4b.2: ANOVA of quercetin 3 O galactoside (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT	3	45677.	15226.	1.49	0.289
out_turn	2	19806.	9903.	0.97	0.420
Residual	8	81738.	10217.		
Total	13	147222.			

A.5.4b.3: ANOVA of quercetin 3 O glucoside (µg g^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT	3	17418.	5806.	0.30	0.821
out_turn	2	12024.	6012.	0.32	0.738
Residual	8	152302.	19038.		
Total	13	181744.			

APPENDIX A.5.5a: Analysis of variance (ANOVA) of total carotenoids of postharvest mango cv. Willard ripened at 30°C and 20°C with temperature variations (µg g^{-1} FW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT	4	6965.1	1741.3	9.79	<.001
Residual	16	2847.0	177.9		
Total	20	9812.1			

APPENDIX A.5.5b: Analysis of variance (ANOVA) of total carotenoids of postharvest mango cv. Karutha Colomban ripened at 30°C and 20°C with temperature variations (µg g^{-1} FW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
CT	3	5897.248	1965.749	267.45	<.001
out_turn	2	246.913	123.457	16.80	<.001
Residual	9	66.150	7.350		
Total	14	6210.311			

APPENDIX A.6: Analysis of variance (ANOVA) of aroma volatile compounds of postharvest mango ripened at 32°C

A.6.1: ANOVA of 3-carene (µg kg^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	6369.0	3184.5	15.31	<.001
out_turn	2	3449.4	1724.7	8.29	0.001
Tissue	1	169.4	169.4	0.81	0.373
Cultivars.out_turn	4	7031.0	1757.7	8.45	<.001
Cultivars.Tissue	2	1318.2	659.1	3.17	0.054
out_turn.Tissue	2	3057.8	1528.9	7.35	0.002
Cultivars.out_turn.Tissue	4	6358.3	1589.6	7.64	<.001
Residual	36	7489.2	208.0		
Total	53	35242.3			

A.6.2: ANOVA of myrcene (µg kg^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	167939519.	83969759.	65.77	<.001
out_turn	2	10131633.	5065817.	3.97	0.028
Tissue	1	17867153.	17867153.	14.00	<.001
Cultivars.out_turn	4	21272717.	5318179.	4.17	0.007
Cultivars.Tissue	2	33372397.	16686199.	13.07	<.001
out_turn.Tissue	2	18006694.	9003347.	7.05	0.003
Cultivars.out_turn.Tissue	4	36080298.	9020075.	7.07	<.001
Residual	36	45960141.	1276671.		
Total	53	350630552.			

A.6.3: ANOVA of ocimene (µg kg^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	48418729.	24209364.	18.60	<.001
out_turn	2	571388.	285694.	0.22	0.804
Tissue	1	31143086.	31143086.	23.92	<.001
Cultivars.out_turn	4	11645296.	2911324.	2.24	0.084
Cultivars.Tissue	2	13548495.	6774248.	5.20	0.010
out_turn.Tissue	2	5297583.	2648792.	2.03	0.146
Cultivars.out_turn.Tissue	4	13136661.	3284165.	2.52	0.058
Residual	36	46869143.	1301921.		
Total	53	170630382.			

A.6.4: ANOVA of terpinolene (µg kg^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	10967959.	5483980.	154.60	<.001
out_turn	2	1380784.	690392.	19.46	<.001
Tissue	1	1593314.	1593314.	44.92	<.001
Cultivars.out_turn	4	2751270.	687818.	19.39	<.001
Cultivars.Tissue	2	3193006.	1596503.	45.01	<.001
out_turn.Tissue	2	564454.	282227.	7.96	0.001
Cultivars.out_turn.Tissue	4	1127920.	281980.	7.95	<.001
Residual	36	1276997.	35472.		
Total	53	22855704.			

A.6.5: ANOVA of humulene (µg kg^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	3595703.	1797851.	101.48	<.001
out_turn	2	48563.	24281.	1.37	0.267
Tissue	1	4061341.	4061341.	229.24	<.001
Cultivars.out_turn	4	451803.	112951.	6.38	<.001
Cultivars.Tissue	2	2742458.	1371229.	77.40	<.001
out_turn.Tissue	2	107565.	53782.	3.04	0.060
Cultivars.out_turn.Tissue	4	611278.	152819.	8.63	<.001
Residual	36	637786.	17716.		
Total	53	12256496.			

A.6.6: ANOVA of α-pinene (µg kg^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	10033468.	5016734.	60.75	<.001
out_turn	2	199950.	99975.	1.21	0.310
Tissue	1	2896525.	2896525.	35.08	<.001
Cultivars.out_turn	4	1617180.	404295.	4.90	0.003
Cultivars.Tissue	2	2001685.	1000843.	12.12	<.001
out_turn.Tissue	2	843286.	421643.	5.11	0.011
Cultivars.out_turn.Tissue	4	2105559.	526390.	6.37	<.001
Residual	36	2972807.	82578.		
Total	53	22670461.			

A.6.7: ANOVA of caryophyllene (µg kg^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	9305267.	4652634.	179.06	<.001
out_turn	2	263999.	132000.	5.08	0.011
Tissue	1	9365739.	9365739.	360.44	<.001
Cultivars.out_turn	4	576935.	144234.	5.55	0.001
Cultivars.Tissue	2	6990875.	3495437.	134.52	<.001
out_turn.Tissue	2	548357.	274179.	10.55	<.001
Cultivars.out_turn.Tissue	4	847726.	211932.	8.16	<.001
Residual	36	935422.	25984.		
Total	53	28834320.			

A.6.8: ANOVA of limonene (μg kg^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	10606.42	5303.21	57.81	<.001
out_turn	2	147.57	73.79	0.80	0.455
Tissue	1	5747.50	5747.50	62.66	<.001
Cultivars.out_turn	4	4370.07	1092.52	11.91	<.001
Cultivars.Tissue	2	3498.03	1749.01	19.07	<.001
out_turn.Tissue	2	1222.20	611.10	6.66	0.003
Cultivars.out_turn.Tissue	4	2812.69	703.17	7.67	<.001
Residual	36	3302.35	91.73		
Total	53	31706.83			

A.6.9: ANOVA of β-pinene (μg kg^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	162167.	81084.	56.40	<.001
out_turn	2	3147.	1573.	1.09	0.346
Tissue	1	34321.	34321.	23.87	<.001
Cultivars.out_turn	4	27353.	6838.	4.76	0.003
Cultivars.Tissue	2	24857.	12428.	8.65	<.001
out_turn.Tissue	2	15074.	7537.	5.24	0.010
Cultivars.out_turn.Tissue	4	34848.	8712.	6.06	<.001
Residual	36	51752.	1438.		
Total	53	353519.			

A.6.10: ANOVA of α-terpinene (μg kg^{-1} DW)

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Cultivars	2	160534.0	80267.0	458.00	<.001
out_turn	2	11366.9	5683.5	32.43	<.001
Tissue	1	48746.7	48746.7	278.14	<.001
Cultivars.out_turn	4	23864.9	5966.2	34.04	<.001
Cultivars.Tissue	2	90139.7	45069.8	257.16	<.001
out_turn.Tissue	2	9698.3	4849.1	27.67	<.001
Cultivars.out_turn.Tissue	4	20419.8	5105.0	29.13	<.001
Residual	36	6309.2	175.3		
Total	53	371079.5			

APPENDIX B

B.1: A book chapter (17) on 'Health promoting-properties of fruits and vegetables' edited Dr. Leon Terry has been published. CAB International 2011, page 352-370.

17 Tropical Fruit [Banana, Pineapple, Papaya and Mango]

Thiruchelvam Thanaraj and Leon A. Terry

17.1 Introduction

Tropical fruit crops (banana, pineapple, papaya and mango) are a group of botanically unrelated crops. They are grouped together for ease in this chapter merely because of their tropical adaptation in the world as major fruit crops. In addition, tropical crops form a substantial part of the export economy of several developing countries. Banana, pineapple and mango are categorized as major tropical fruit crops, while papaya is considered as a minor crop (Galán Saúco, 1996).

Tropical fruit are commonly consumed fresh. Despite their relatively low calorific value (banana and plantain are the exceptions), tropical fruit play a major role in the human diet, mainly because of their high and diverse concentration of vitamins, minerals, carotenoids and other bioactive components. The antioxidant activities of tropical fruit have been discussed in a few studies only, even though some of them are rich in dietary antioxidants (Jimenez-Escrig *et al.*, 2001; Bashir *et al.*, 2003; Bennett *et al.*, 2010). Mango and papaya are a good source of carotenoids (β-carotene) and vitamin C, while banana is rich in polyphenols (Arora *et al.*, 2008; Vijayakumar *et al.*, 2008; Bennet *et al.*, 2010). The pineapple is rich in vitamin C (Cordenunsi *et al.*, 2010). However, pineapple and papaya contain proteolytic enzymes, namely bromelain and papain, respectively. These enzymes have significant chemopreventive activities and are used in several industrial applications. Tropical fruit also contain high levels of pectin, fibre and cellulose, which are believed to promote intestinal motility. The relatively high organic acid content of many tropical fruit may also stimulate appetite and aid digestion (Martin *et al.*, 1987).

In the main, tropical fruit are rich in health-promoting properties and are associated with several health benefits to humans; however, the published information is still very limited. Therefore, it is necessary to highlight the health benefits of tropical fruit, which may improve awareness among people, especially in the developing world, in selecting and consuming these fruit.

17.2 Banana

17.2.1 Introduction

Banana is a common name for the fruit of the genus *Musa*, which bears edible fruit of the family Musaceae. Edible fruit-bearing banana cultivars belong to the species endemic to the South-east Asian origin (*Musa acuminata* and *M. balbisiana*) (Zhang *et al.*, 2005). Banana usually refers to a soft, sweet dessert fruit; however, the green, firm and starchy fruit are from

B.2: A Thanaraj *et al.* (2009). A paper published in Food Chemistry

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Chemometric profiling of pre-climacteric Sri Lankan mango fruit (*Mangifera indica* L.)

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ABSTRACT

There is no published information on the genotypic variation of major biochemical constituents in mango fruit endemic to Sri Lanka. Accordingly, non-structural carbohydrates, non-volatile organic acids and total phenolics were determined from the peel and pulp of pre-climacteric Sri Lankan mango cultivars (*viz.* Willard, Karutha Colomban, Vellai Colomban, Ampalavi, and Malgova) at three different maturity stages. Principal components analysis revealed distinct clustering of samples according to their biochemical profiles of peel and pulp at three maturity stages. Sugar concentrations generally declined with maturity in both peel and pulp except for cv. Willard. Fructose was the predominant sugar in both peel (56.2–106 mg/g dry weight (DW)) and pulp (67.4–141 mg/g DW), followed by glucose and sucrose. Starch concentration increased with maturity and was higher in pulp (26.0–55.0% DW) than peel (18.2–38.9% DW) at full mature stage. Dry matter as a proportion of fresh weight (FW) increased with maturity.

B.3: A paper is published in Acta Horticulturae following an oral presentation made at the postharvest unlimited conference during November 4 – 7, 2008 at Berlin

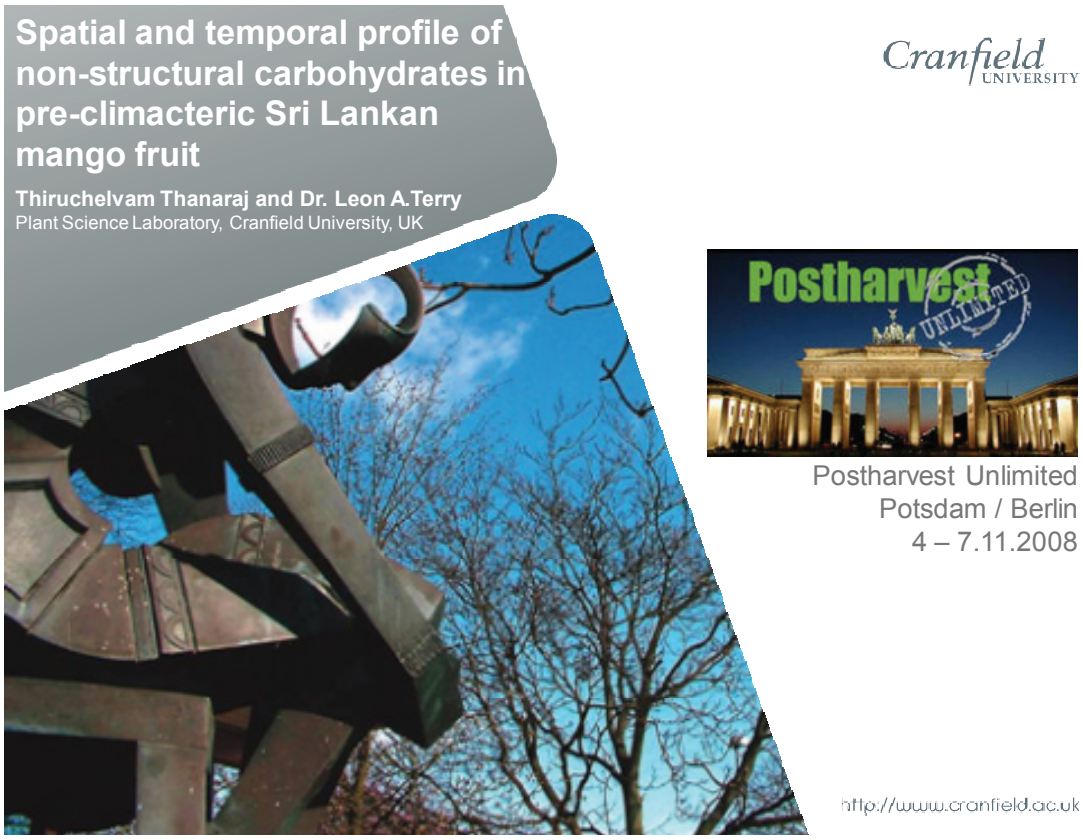
ISHS Acta Horticulturae 858: III International Conference Postharvest Unlimited 2008

SPATIAL AND TEMPORAL PROFILE OF NON-STRUCTURAL CARBOHYDRATES IN PRE-CLIMACTERIC SRI LANKAN MANGO (*MANGIFERA INDICA* L.) FRUIT

Authors: T. Thanaraj, L.A. Terry

Keywords: chemical composition, maturity stages, peel, pulp, starch, sugars

Abstract:
There is no published information on the genotypic variation of major biochemical constituents in mango fruit endemic to Sri Lanka. Accordingly, non-structural carbohydrates (NSCs) were determined from the peel and pulp of pre-climacteric Sri Lankan mango cultivars (viz. 'Willard', 'Karutha Colomban' and 'Malgova') at three different maturity stages. Sugars and starch were quantified using standard HPLC and a total starch assay kit, respectively. Sugar content of both pulp and peel reduced with maturity in 'Malgova' and 'Karutha Colomban', yet increased in 'Willard'. Total sugars were significantly higher in the pulp and peel (300.7 mg g^{-1} and 177.1 mg g^{-1}, respectively) of 'Malgova' than that of 'Willard' (236.5 mg g^{-1} and 143.2 mg g^{-1}) and 'Karutha Colomban' (128.1 mg g^{-1} and 85.4 mg g^{-1}). Reducing sugars contributed to ca. 80% of total sugars, whereby fructose was dominant in both pulp (67.4-141.3 mg g^{-1}) and peel (56.2-106.1 mg g^{-1}) followed by glucose and sucrose. Sucrose (5.2-29.8 mg g^{-1}) was significantly lower in peel samples. Although there was no noticeable variation in starch concentration between pulp and peel at immature stage, starch increased with maturity and was significantly higher in pulp (26.0-55.0%) than peel (18.2-38.9%) at full mature stage. The mean starch concentration was higher in both pulp (36.6 mg g^{-1}) and peel (31.2 mg g^{-1}) of 'Malgova' followed by 'Karutha Colomban' and 'Willard'. Implications of these biochemical changes on subsequent postharvest quality are discussed.



B.4: A paper is published in Acta Horticulturae following an oral presentation made at the 6th International Postharvest symposium during April 8 – 12, 2009 at Antalya, Turkey

ISHS Acta Horticulturae 877: VI International Postharvest Symposium

TEMPORAL CHANGE IN TASTE- AND HEALTH-RELATED COMPOUNDS DURING POSTHARVEST RIPENING OF SRI LANKAN MANGO FRUIT (*MANGIFERA INDICA* L.)

Authors: T. Thanaraj, L.A. Terry

Keywords: sugars, organic acids, flavonoids, climacteric, ascorbic acid, total phenolics

Abstract:
The taste preference and health benefits of ripe mango fruit predominantly depend on biochemical changes during postharvest ripening. Therefore, understanding the variation of biochemical compounds (sugars, organic acids, total titratable acidity (TTA), total soluble solids (TSS), flavonoids, total phenolics (TP) and total carotenoids) during ripening may help to optimise the ripening period. Sri Lankan mango fruits ('Karutha Colomban', 'Malgova' and 'Willard') were ripened at 32°C for 4 days and both peel and pulp sample

were analysed. Sucrose (65-85% in pulp and 40-50% in peel) and citric acid (88.5% in pulp and 60% in peel) were the main components in total sugar and organic acid compositions, respectively. The increase in sugar and decrease in organic acids during ripening increased the sugar/acid ratio. Ascorbic acid (AsA) was significantly higher in 'Willard' and dominated in peel samples. Total phenolics decreased during ripening and were about ten-fold higher in peel than pulp. A significant increase was observed in total carotenoids during ripening and 'Karutha Colomban' and 'Willard' had about two-fold higher concentration than 'Malgova'. Mango 'Willard' had significantly higher concentration of flavonoids than other cultivars, mangiferin was the dominant flavonoid in cultivars tested followed by quercetin 3-O galactoside and quercetin 3-O glucoside.

B.5: A paper is published at the proceeding of the Annual Research Conference following a poster presentation made at the 7th Annual Research Conference of Eastern University, Sri Lanka during November 20 – 23, 2008

EFFECT OF RIPENING ON CHEMOMETRIC PROFILE OF TASTE-RELATED COMPOUNDS IN MANGO CV. WILLARD FRUIT

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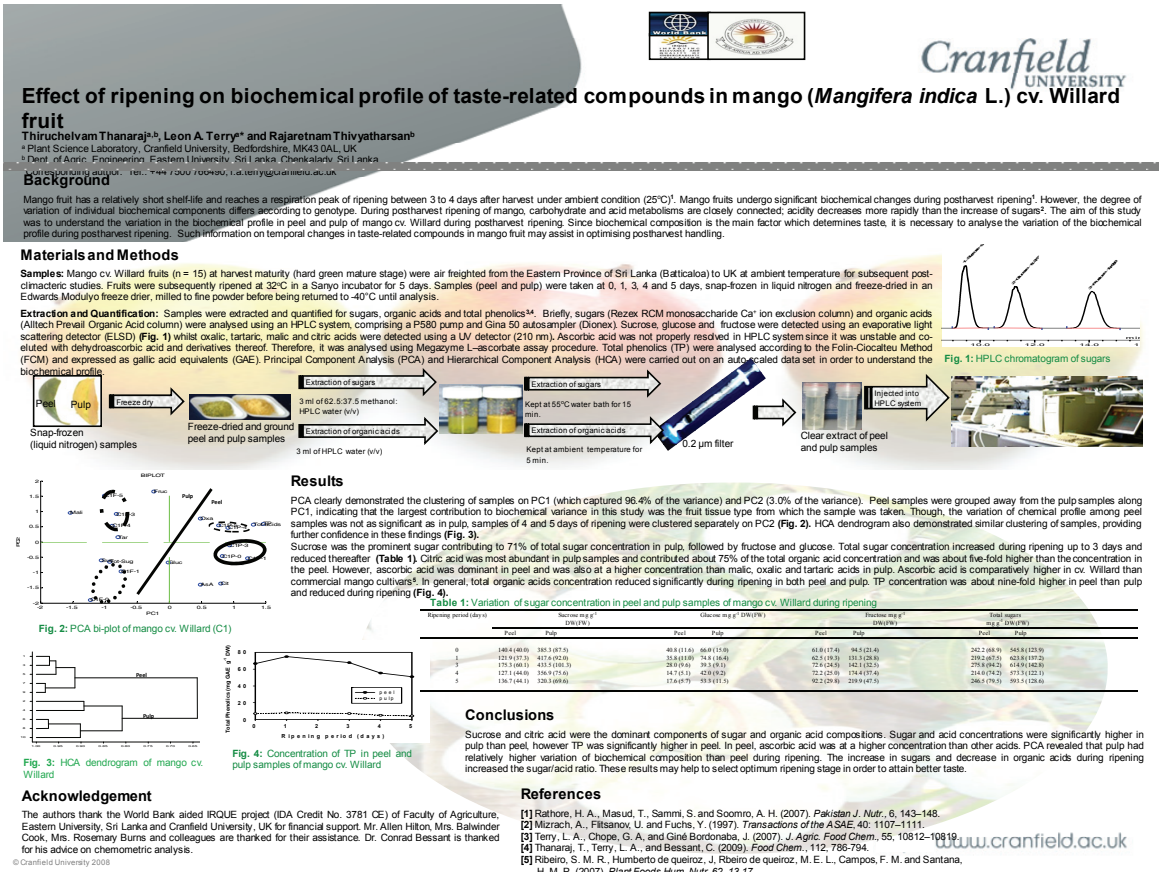
ABSTRACT

Mango (*Mangifera indica* L.) is one of the important climacteric fruit cultivated in most tropical countries. Both the physiological and biochemical changes during ripening are marked. Non-structural carbohydrates, non-volatile organic acids (OA) and total phenolics (TP) were determined from the peel and pulp samples of post-climacteric mango cv. Willard during the 5 days of ripening at 32°C. Explanatory data analysis using principal components analysis (PCA) and hierarchical cluster analysis (HCA) revealed distinct



Effect of ripening on biochemical profile of taste-related compounds in mango (*Mangifera indica* L.) cv. Willard fruit

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Background

Mango fruit has a relatively short shelf-life and reaches a respiration peak of ripening between 3 to 4 days after harvest under ambient condition (25°C)[1]. Mango fruits undergo significant biochemical changes during postharvest ripening[1]. However, the degree of variation of individual biochemical components differs according to genotype. During postharvest ripening of mango, carbohydrate and acid metabolisms are closely connected; acidity decreases more rapidly than the increase of sugars[2]. The aim of this study was to understand the variation in the biochemical profile in peel and pulp of mango cv. Willard during postharvest ripening. Since biochemical composition is the main factor which determines taste, it is necessary to analyse the variation of the biochemical profile during postharvest ripening. Such information on temporal changes in taste-related compounds in mango fruit may assist in optimising postharvest handling.

Materials and Methods

Samples: Mango cv. Willard fruits (n = 15) at harvest maturity (hard green mature stage) were air freighted from the Eastern Province of Sri Lanka (Batticaloa) to UK at ambient temperature for subsequent post-climacteric studies. Fruits were subsequently ripened at 32°C in a Sanyo incubator for 5 days. Samples (peel and pulp) were taken at 0, 1, 3, 4 and 5 days, snap-frozen in liquid nitrogen and freeze-dried in an Edwards Modulyo freeze drier, milled to fine powder before being returned to -40°C until analysis.

Extraction and Quantification: Samples were extracted and quantified for sugars, organic acids and total phenolics[3,4]. Briefly, sugars (Rezex RCM monosaccharide Ca+ ion exclusion column) and organic acids (Alltech Prevail Organic Acid column) were analysed using an HPLC system, comprising a P580 pump and Gina 50 autosampler (Dionex). Sucrose, glucose and fructose were detected using an evaporative light scattering detector (ELSD) **(Fig. 1)** whilst oxalic, tartaric, malic and citric acids were detected using a UV detector (210 nm). Ascorbic acid was not properly resolved in HPLC system since it was unstable and co-eluted with dehydroascorbic acid and derivatives thereof. Therefore, it was analysed using Megazyme L–ascorbate assay procedure. Total phenolics (TP) were analysed according to the Folin-Ciocalteu Method (FCM) and expressed as gallic acid equivalents (GAE). Principal Component Analysis (PCA) and Hierarchical Component Analysis (HCA) were carried out on an auto-scaled data set in order to understand the biochemical profile.

Fig. 1: HPLC chromatogram of sugars

Results

PCA clearly demonstrated the clustering of samples on PC1 (which captured 96.4% of the variance) and PC2 (3.0% of the variance). Peel samples were grouped away from the pulp samples along PC1, indicating that the largest contribution to biochemical variance in this study was the fruit tissue type from which the sample was taken. Though, the variation of chemical profile among peel samples was not as significant as in pulp, samples of 4 and 5 days of ripening were clustered separately on PC2 **(Fig. 2).** HCA dendrogram also demonstrated similar clustering of samples, providing further confidence in these findings **(Fig. 3).**

Sucrose was the prominent sugar contributing to 71% of total sugar concentration in pulp, followed by fructose and glucose. Total sugar concentration increased during ripening up to 3 days and reduced thereafter **(Table 1).** Citric acid was most abundant in pulp samples and contributed about 75% of the total organic acid concentration and was about five-fold higher than the concentration in the peel. However, ascorbic acid was dominant in peel and was also at a higher concentration than malic, oxalic and tartaric acids in pulp. Ascorbic acid is comparatively higher in cv. Willard than commercial mango cultivars[5]. In general, total organic acids concentration reduced significantly during ripening in both peel and pulp. TP concentration was about nine-fold higher in peel than pulp and reduced during ripening **(Fig. 4).**

Fig. 2: PCA bi-plot of mango cv. Willard (C1)

Table 1: Variation of sugar concentration in peel and pulp samples of mango cv. Willard during ripening

Ripening period (day s)	Sucrose mg g^{-1} DW(FW)		Glucose mg g^{-1} DW(FW)		Fructose mg g^{-1} DW(FW)		Total sugars mg g^{-1} DW(FW)	
	Peel	Pulp	Peel	Pulp	Peel	Pulp	Peel	Pulp
0	140.4 (40.0)	385.5 (87.5)	40.8 (11.6)	66.0 (15.0)	61.0 (17.4)	94.5 (21.4)	242.2 (68.9)	545.9 (123.9)
1	321.9 (37.3)	417.6 (92.0)	35.8 (11.0)	74.6 (16.4)	62.5 (19.3)	131.3 (28.8)	419.2 (67.5)	623.8 (197.2)
3	175.3 (60.1)	433.5 (101.3)	28.0 (9.6)	59.3 (9.1)	72.6 (24.5)	142.1 (32.5)	275.9 (94.2)	634.9 (142.8)
4	127.1 (44.0)	336.9 (75.6)	14.7 (5.1)	42.0 (9.2)	72.2 (25.0)	174.4 (37.4)	214.0 (74.2)	277.3 (122.1)
5	136.7 (44.1)	320.3 (69.6)	17.6 (5.7)	53.3 (11.5)	92.2 (29.8)	219.9 (47.5)	246.5 (79.5)	593.5 (128.6)

Fig. 3: HCA dendrogram of mango cv. Willard

Fig. 4: Concentration of TP in peel and pulp samples of mango cv. Willard

Conclusions

Sucrose and citric acid were the dominant components of sugar and organic acid compositions. Sugar and acid concentrations were significantly higher in pulp than peel, however TP was significantly higher in peel. In peel, ascorbic acid was at a higher concentration than other acids. PCA revealed that pulp had relatively higher variation of biochemical composition than peel during ripening. The increase in sugars and decrease in organic acids during ripening increased the sugar/acid ratio. These results may help to select optimum ripening stage in order to attain better taste.

Acknowledgement

The authors thank the World Bank aided IRQUE project (IDA Credit No. 3781 CE) of Faculty of Agriculture, Eastern University, Sri Lanka and Cranfield University, UK for financial support. Mr. Allen Hilton, Mrs. Balwinder Cook, Mrs. Rosemary Burns and colleagues are thanked for their assistance. Dr. Conrad Bessant is thanked for his advice on chemometric analysis.

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B.6: A poster presentation made at the Annual Postgraduate Conference of Cranfield Health, Cranfeild University in 2008.

Chemometric profiling of pre-climacteric Sri Lankan mango fruit (*Mangifera indica* L.)

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Background

Mangoes should be harvested at a physiologically mature, hard, green stage for subsequent postharvest ripening. However, harvesting criteria vary according to local consumption patterns and distance to market. Mature hard green mangoes attain a superior eating quality when ripe whilst immature ones do not; thus measuring the harvest quality of hard green mango fruit is very important in order to discriminate immature from mature fruits at harvest[1]. Starch is the major carbohydrate present in mature green mangoes and is hydrolysed into sugars during ripening[2]. The aim of this study was to improve the understanding of spatial distribution and temporal changes in chemical composition of pre-climacteric Sri Lankan mangoes using chemometric analysis, which may assist in predicting optimum harvest maturity.

Materials and Methods

Samples: Five prominent mango cultivars, endemic to Sri Lanka (Willard, Karutha Colomban, Vellai Colomban, Ampalavi and Malgova) were selected at three different maturity stages *viz.* immature, half mature and full mature (130-140 days after full bloom (DAFB)) from the Eastern University, Sri Lanka. Mangoes were selected randomly in three replicates at each maturity stage based on DAFB, fruit shape and maturity of seed stone. Peel and pulp samples from the stem, middle and distal ends of a fruit were snap-frozen in liquid nitrogen immediately after the harvest and stored at -20°C. Samples were air freighted in dry ice to Plant Science Laboratory, Cranfield University and then freeze dried.

Extraction and Quantification: Samples were extracted and quantified for sugars, organic acids, starch and total phenolics[3,4]. Briefly, sugars (Rezex RCM monosaccharide Ca+ size exclusion column) and organic acids (Alltech Prevail Organic Acid column) were analysed using a HPLC system, comprising a P580 pump and Gina 50 autosampler (Dionex). Sucrose, glucose and fructose were detected using evaporative light scattering detector (ELSD) **(Fig. 1)** whilst oxalic, tartaric, malic and citric acids were detected using UV detector (210 nm) **(Fig. 2)**. Ascorbic acid was not properly resolved since it was unstable and coeluted with dehydroascorbic acid and derivatives thereof.

Results

Principal components analysis of cv. Willard clearly demonstrated the clustering of samples on PC1 and PC2. Peel samples were grouped away from the pulp samples along PC1, indicating that the largest contribution to biochemical variance in this study was the fruit tissue type (peel and pulp) from which the sample was taken. Although pulp samples could not be discriminated along PC1, the samples were grouped separately into immature, fully mature and half mature pulp samples on PC2. Data from the other tested cultivars were also separated into clusters, however the clustering was not as distinct as for cv. Willard **(Fig. 3)**.

In general, total sugar concentration declined significantly from immature stage (199 mg g^{-1} DW) to fully mature stage (162 mg g^{-1} DW) of mango **(Fig. 4)**. Starch levels varied significantly between fully mature (38.6% DW) and immature (19.7% DW) stages **(Fig. 5)**. Total acidity reduced with maturity (178 – 97.5 mg g^{-1} DW) **(Fig. 6)**. There were no significant differences ($P > 0.05$) in total phenolics among mango cultivars, maturity stages and fruit tissue type.

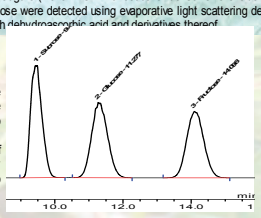

Fig. 1: HPLC chromatogram of sugars from mango fruit

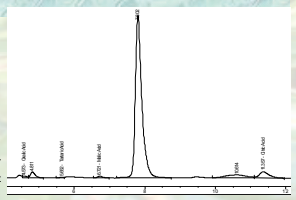

Fig. 2: HPLC chromatogram of organic acids from mango fruit

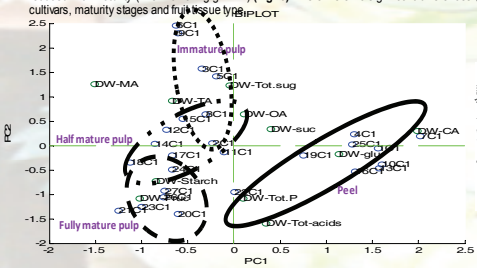

Fig. 3: PCA bi-plot for PC1 (72.65%) versus PC2 (22.93%) of pre-climacteric mango cv. Willard (C1). Samples (1C–27C; n = 27) from peel and pulp of mango fruits at different maturity stages.

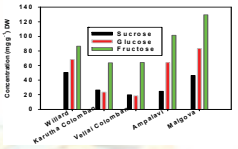

Fig. 4: concentration of sugars in the pulp samples of different mango cultivars

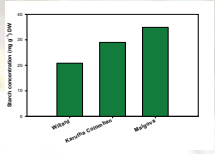

Fig. 5: Starch concentration in the pulp samples of different mango cultivars

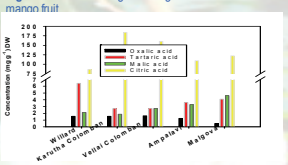

Fig. 6: Concentration of organic acids in the pulp samples of different mango cultivars

Conclusions

Fully mature mango fruit (130–140 DAFB) had the highest concentration of starch and lowest concentration of sugars and acids. Chemometric analysis clearly revealed distinct differences according to cultivar, tissue type and maturity, based upon non-structural carbohydrates, acids and total phenolics. Since these combined variables are partially responsible for the final quality of ripe fruit, they could be used to harvest pre-climacteric Sri Lankan mango fruit at the optimum stage for subsequent postharvest ripening.

Acknowledgement

The authors thank the World Bank aided IRQUE project (IDA Credit No. 3781 CE) of Faculty of Agriculture, Eastern University, Sri Lanka and Cranfield University, UK for financial support. Mr. Allen Hilton, Mrs. Balwinder Cook and Mrs. Rosemary Burns are thanked for their technical assistance.

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B.7: A paper is about to be submitted to the Journal of Food Composition and Analysis.

Temporal change in biochemical profile of Sri Lankan mango fruits during postharvest ripening

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Abstract

Mango fruit (*Mangifera indica* L.) is popular in the tropics for its characteristic aroma and taste. In addition to pulp tissue, mango peel (major bi-product in mango industry) is also a good source of dietary antioxidants. However, a little is known about the composition of bioactive compounds in Sri Lankan mango cultivars. Mango cultivars endemic to Sri Lanka *viz*. Willard, Karutha Colomban and Malgova fruits were air freighted from Sri Lanka to the UK at harvest maturity and ripened for 4 days at 32^{o}C. Peel and pulp samples were obtain at day 0, 3 and 4 during ripening, extracted and analysed for sugars, non-volatile organic acids, total titratable acidity (TTA), AsA, total phenolics (TP), individual flavonoids and total carotenoids.

B.8: An abstract was accepted for an oral presentation at the International Mango Symposium, China.

Thiruchelvam Thanaraj and Leon A. Terry (2010). Temporal variation of aroma volatile compounds from Sri Lankan mango fruit during ripening at different temperatures. International Mango Symposium, China (http://www.mango2010.cn/english.html).

B.9: An abstract was accepted for an oral presentation at the Fav Health 2009, France.

Thiruchelvam Thanaraj and Leon A. Terry (2009). Health Promoting Properties of Sri Lankan Mango Fruits (S9SC5). 3rd International Symposium on Human Health Effects of Fruits and Vegetables, Fav Health 2009, October 18-21, Avignon, France. (http://www.cdiem-events.com/favhealth2009/pdf/ORAL_prog.pdf).

Health promoting properties of Sri Lankan mango fruits

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Abstract

Mango fruit (*Mangifera indica* L.) is popular in tropics for its characteristic aroma and taste, however it is also a good source of dietary antioxidants such as ascorbic acid (AsA), carotenoids and phenolic compounds. The antioxidants play an important role in reducing the risk of degenerative diseases and offer several health benefits to human. However, antioxidant capacity of fruits varies with genotype, harvest maturity, harvesting season, postharvest storage conditions and processing. Mango peel (major bi-product in mango industry) is also a good source for phenolic compounds, ascorbic acid, carotenoids and other bioactive compounds.

Sri Lankan mango cvs. Willard and Karutha Colomban fruits were air freighted from Sri Lanka at harvest maturity and ripened for 6 days at 20°C and 30°C at the Plant Science Laboratory, Cranfield University. Peel and pulp samples were obtained at day 3 and day 6 (n = 3) and extracted and analysed for AsA, total phenolics (TP), flavonoids and total carotenoids. Mango cv. Willard contained significantly higher concentration of AsA, TP and flavonoids than cv. Karutha Colomban. However, total carotenoids concentration was higher in cv. Karutha Colomban than cv. Willard. Ascorbic acid concentration increased up to day 3 and then decreased, however the decreasing trend was higher in fruits ripened at 30°C. A significant increase was observed in total carotenoids during ripening, however the concentration was relatively higher in fruits ripened at 30°C. Mangiferin was the dominant flavonoid in cultivars tested and followed by quercetin 3-O galactoside and quercetin 3-O glucoside. However, magiferin concentration increased during ripening in cv. Willard whilst decreased in cv. Karutha Colomban irrespective of ripening temperatures.

Keywords: ascorbic acid; carotenoids; flavonoids; total phenolic; bioactive compounds; antioxidants

APPENDIX C: Chapter 7 Tables

Appendix C 7.1 Sugars concentration of different mango cultivars extracted and analysed in different methods

Analytes	Extraction Method	Fruit maturity/ Section	Cultivar	Quantification Method	Range Measured	Reference
Sucrose, glucose and fructose (mg g^{-1} FW)	According to Hubbard *et al.,* 1990, Blackeney and Mutton (1980)	Ripe fruit (pulp)	Haden (Venezuela)	Lane and Eynon's method (AOAC, 1984)	Sucrose-110, Fructose-44, glucose-5	Castrillo *et al*., 1992
Sugars (Spongy tissues) (mg g^{-1} FW)	50 ml of distilled water with 0.5 ml of 0.5N NaOH and neutralised with 0.5N HCL, 5ml of filtrate mxed with 20 ml of 85% EtOH	Mature (pulp)	Tommy Atkins (Brazil)	Calculated from the standard D-glucose calibration curve.	Reducing sugars: 15 Non reducing sugar: 39	Lima *et al.,* 2001
Sucrose, glucose and fructose (mg g^{-1} FW)	According to Ueda *et al*., 1999	Ripe fruit (pulp)	Chiin Hwang No.1 (Japan)	HPLC using a µBondapak/carbohydrate analytical column.	Sucrose-120, Fructose-30, glucose-16	Ueda *et al*., 2001
Total measured sugars - fructose, glucose and sucrose (mg g^{-1} FW)	Fresh samples suspended in 10 ml of water and filtered, then injected into HPLC model 10AD	Mature (pulp)	Mahajanaka (Thailand)	Column: Shimpack SCR-10N; Mobile phase: water; Flow rate: 0.8 ml min^{-1}; Column Temp.: 60°C; Detector: RI	Total measured sugars:60.06 (105 DAFS), 63.64 (140 DAFS) Fructose: 30.03 (105 DAFS), 31.09 (140DAFS) Glucose: 2.93 (105 DAFS), 1.63 (140 DAFS) Sucrose: 27.1 (105 DAFS), 30.91 (140 DAFS)	Saranwong *et al.,* 2004
Total measured sugars- fructose, glucose and sucrose (mg g^{-1} DW)	Lane and Eynon method (No further details)	Fully ripe (pulp)	Baneshan, Suvarnarekha and Totapuri (India)	No details	Total measured sugars: 357.25 Fructose+glucose: 49.8 Sucrose: 307.45	Hymavathi and Khader, 2005

Total measured sugars - fructose, glucose and sucrose (mg g^{-1} DW)	AOAC,1995 method; Lalel *et al*, 2003b after the 24 days storage period.	Ripe fruit (pulp)	Delta R2E2 (Australia)	No details	Total measured sugars: 208.8 Fructose + glucose: 95.1 Sucrose: 113.7	Lalel *et al*, 2005
Total measured sugars - fructose, glucose and sucrose (mg g^{-1} FW)	Association of Official Analytical Chemists (AOAC, 1996) method with some modifications (Singh *et al.*, 2000).	Ripe fruit (pulp)	Kensington Pride (Australia)	Sugars were quantified on an amino-bonded column with a mobile phase of CH_3CN and H_2O (80/20 v:v) and detection with a deferential refractometer. Using HPLC, according to Ueda *et al*., 1999	Total measured sugars: 159 Fructose + glucose: 43 Sucrose-116	Malik and Singh, 2006
Total measured sugars - fructose, glucose and sucrose (mg g^{-1} FW)	Alcohol soluble sugars were extracted with 70% ethanol and later purified by passage through Dowex 50 (H+) and Dowex 1 (OH-) columns.	Unripe and ripe (pulp)	Alphonso (India)	Determined by Phenol-H_2SO_4, (Gilles *et al.*, 1956). Analyzed in HPLC fitted with LC-6A pump, Shimpak C_{18} column (4.6mm× 250 cm) eluted with acetonitrile–water (70:30, v/v, at a flow rate of 0.6mL min^{-1} at 40 °C) and the elution was monitored by RI detector set at 8×10^{-6}RIU.	Unripe - Total measured sugars: 10.1 Fructose: 6.1 Glucose: 3.8 Sucrose: 0.2 Ripe - Total measured sugars: 138 Fructose: 49 Glucose: 39 Sucrose: 50	Yashoda *et al.,* 2006
Sucrose, glucose and fructose (mg g^{-1} FW)	According to Smith *et al*., (1986). 10 g fresh pulp tissue extracted in 50 ml water.	Ripe fruit (pulp)	Ataulfo (A), Kent (Ke), Keitt (Ki) (Mexico)	According to Hubbard *et al.,* 1990, Blackeney and Mutton (1980) and Tawfik and Mardon (1985)	Sucrose-120(A),70(Ke), 87(Ki) Fructose-18(A),30(Ke), 28(Ki) glucose-6(A),5(Ke), 1(Ki)	Gonzalez-Aguilar *et al*., 2008
Total and reducing sugars (mg g^{-1} FW)	Lane and Eynon's method (AOAC, 1984)	Ripe fruit (pulp)	Cat Hoa Loc (Vietnam)	Lane and Eynon's method (AOAC, 1984)	Total measured sugars – 141.5, reducing sugars – 22.1	Hoa and Ducamp, 2008

Total and reducing and non-reducing sugars (mg g^{-1} FW)	Not mentioned.	Ripe fruit (pulp)	Samar Bahisht Chaunsa (Pakistan)	Not mentioned.	Sucrose-179.2, Reducing sugars-28.3 and total measured sugars-207.1	Amin *et al.*, 2009
Sucrose, glucose and fructose (mg g^{-1} DW)	According to Thanaraj *et al*., 2009.	Ripe fruit (pulp)	Anwar Rotale (AR), Faiz Kareem (FK) and Chaunsa (C), (Venezuela)	According to Thanaraj *et al*., 2009	Sucrose: 586 (AR), 511.6 (FK), 593 (C) Fructose: 99.7 (AR), 61.9 (FK), 22.9 (C) Glucose:30.2 (AR), 5.5 (FK), 2.1 (C)	Rajwana *et al*., 2010

NaOH: Sodium hydroxide
AOAC: Association of official analytical chemist
EtOH: Ethanol
HPLC: High performance liquid chromatography

Appendix C 7.2 Starch concentration of different mango cultivars extracted and analysed in different methods

Analytes	Extraction Method	Fruit maturity/ Section	Cultivar	Quantification Method	Range Measured	Reference
Starch (% DW)	250 g of fruit pulp died at 70°C (Lustre *et al*., 1976)	Mature (pulp)	Carabao (Philippines)	According to Lustre *et al*., 1976	11.41	Morga *et al*., 1979
Starch (% DW)	A modification of mtd. of Nielsen (1943) was followed as described (Pesis *et al*., 1978)	Mature (pulp)	Haden (Israel)	A modification of mtd. of Nielsen (1943) was followed as described (Pesis *et al*., 1978)	21	Fuchs *et al*., 1980
Starch (% DW)	According to Hubbard *et al*., (1990). 1 g of frozen pulp samples homogenised in 5 ml of boiling 80% EtOH (v/v).	Mature (pulp)	Haden (Venezuela)	According to Hubbard *et al*., (1990).	40	Castrillo *et al*., 1992
Starch (% DW)	Residues after the extraction of sugars re-suspended with amiloglucosidase solution of 14U ml^{-1} in 0.1N sodium acetate buffer, pH 4.8 for 2 h at 40°C.	Mature (pulp)	Tommy Atkins (Brazil)	Reducing sugars produced determined from the standard D-glucose calibration curve.	11.2	Lima *et al.,* 2001
Starch (% DW)	Extracted from alcohol insoluble solids of mango fruit pulp.	Mature (pulp)	Chiin Hwang No.1 (Japan)	Liberated free reducing sugars were estimated using Somogyi-Nelson method	50	Ueda *et al*., 2001
Starch (% DW)	5cm diameter and 10 mm deep pulp portion of mango dried at 70°C for 48 h and extracted for the analysis in total starch assay kit (Megazyme)	Mature (pulp)	Mahajanaka (Thailand)	Determined against the D-glucose standard in spectrophotometer.	46.31 (105DAFS) 55.17 (140DAFS)	Saranwong *et al.,* 2004

Starch (% DW)	Determined by enzymatic hydrolysis by glucose (Lechaudel *et al*., 2005)	Mature (pulp)	Cogshall (France)	Determined by enzymatic hydrolysis by glucose (Lechaudel *et al*., 2005)	24	Lechaudel and Joas, 2006
Starch (% DW)	50 mg of sample was dispensed in 2M KOH for 30 min to hydrolyse all starch then incubated with amyloglucosidase.	Mature green preclimacteric mango (Peel and pulp)	Tommy Atkins (Mexico)	Glucose was determined using glucose oxidase assay GOD-POD.	29.88	Vergara-Valencia *et al.,* 2007
Starch (% DW)	According to Cordenunsi and Lajolo, 1995	Mature (pulp)	Keitt (Brazil)	According to Cordenunsi and Lajolo, 1995	21	Simao *et al*., 2008

EtOH: Ethanol

Appendix C 7.3 Non-volatile organic acids concentration of different mango cultivars extracted and analysed in different methods

Analytes	Extraction Method	Fruit maturity/ Section	Cultivar	Quantification Method	Range Measured	Reference
Vitamin C (mg g^{-1} FW)	10 g of peel and pulp tissue homogenized with 100 ml of 3% metaphosphoric acid-acetic acid reagent.	Fully ripe pulp Fully ripe peel	Alphonso (A) Dasheri (D) Langra (L) Pairi (P) (India)	Estimated by visual titration against 2,6-dichlorophenol indophenols dye (Association of Vitamin Chemists, 1966)	1.03(A), 0.3(D), 1.14 (L), 0.4(P) 2.19(A), 1.3(D), 5.46 (L), 2.5(P)	Thomas and Oke, 2007
Citric, oxalic, ascorbic, malic, tartaric and total acids (mg g^{-1} FW)	10 g fresh pulp was blended and homogenized by omni-mixer and refluxed for 15 min and centrifuged for 30 min , residues was washed with 20 ml MeOH twice and dried at 35^{o}C and then redisolved in 50 ml distilled water.	Ripe fruit pulp	Lippens (L) Smith (S) (Spain)	HPLC equipped with Hawlett-Packard Model 1040 quatenary solvent delivery system with C-18 column (small) and rapid-scanning UV/visible photodiode array detector.	Oxalic acid – 0.07 (L), 0.06 (S) Citric acid – 6.26 (L), 8.88 (S) Tartaric acid – Traces (L), (S) AsA – 0.35 (L), 0.26 (S) Malic acid – 1.68 (L), 1.62 (S) Total acids – 8.89 (L), 11.27 (S)	Cano *et al*., 1994
AsA (mg g^{-1} FW)	Visual titration with 2,4-dichlorophenol indophenols dye.	Ripe fruit (pulp)	Alphonso (India)	Visual titration with 2,4-dichlorophenol indophenols dye.	0.26	Padmini and Prabha, 1997
Citric and malic acids (mg g^{-1} FW)	According to Ueda *et al*., 2000	Ripe fruit (pulp)	Chiin Hwang No.1 (Japan)	Using HPLC, according to Ueda *et al*., 2000	Citric acid-6, malic-2.5	Ueda *et al*., 2001
Ascorbic acid (mg g^{-1} DW)	Xylene extraction method (No further details).	Fully ripe (pulp)	Baneshan (B), Suwarnarekha (S) and Totapuri (T) (Indian)	No details	0.348(B), 0.2658(S)	Hymavathi and Khader, 2005
Ascorbic acid (mmol kg^{-1}DW)	Extracted in 10% (w/v) metaphosphoric acid.	Peel and Pulp	Choke anan (Thailand)	Analysed by 2, 4-dinitrophenylhydrazine method by Kondo *et al.,* 2002a.	14 DAFB:0.28(peel), 1.05(pulp) 56 DAFB:0.58(peel), 0.69(pulp) 84 DAFB:0.79(peel), 0.72(pulp)	Kondo *et al.,* 2005

Ascorbic acid (mg g^{-1} DW)	No details	Unripe and ripe (peel)	Raspuri (R) Badami (B) (India)	Method of Omaye *et al.* (1973), ascorbic acid used as standard.	Unripe: 0.188 (R), 0.315 (B) Ripe: 0.349 (R), 0.392 (B)	Ajila *et al.,* 2007a
AsA (mg g^{-1} FW)	E1-titrimetry, E1-LC, E2-LC	Un ripe pulp Half ripe pulp Fully ripe pulp	Keitt (Brazil)	LC with UV-visible detection and Shodex RSpak KC-811 column	0.8(E1-T),0.76(E1-LC),0.84(E2-LC) 0.6(E1-T),0.61(E1-LC),0.58(E2-LC) 0.6(E1-T),0.54(E1-LC),0.39(E2-LC)	Hernandez-Guerra *et al.,* 2006
Total acidity AsA (mg g^{-1} DW)	AOAC, 1996 standard methods (Jagota and Dani, 1982). Adding 6% metaphosphoric acid solution containing 0.18% of ethylenediaminetetraacetic acid (EDTA).	Fruit (pulp)	Kensington Pride (Australia)	UV- visible spectrophotometer at 760 nm.	Ascorbic acid: 0.25 Total acidity: 2	Malik and Singh, 2006
Vitamin C (mg g^{-1} FW)	No details	Fully ripe pulp	Keitt (Ki) Kent (Ke) Tommy Atkins (TA) (Brazil)	According to Jemey and Kovacs (1968)	0.33 (Ki), 0.32 (Ke), 0.26 (TA)	Mansour *et al*., 2006
Citric acids Malic acids Succinic acids (mg g^{-1} DW)	No details	Unripe and Ripe (pulp)	Alphonso (India)	No details	Unripe - Citric: 24.8 Malic: 0.3 Succinic: 2.9 Ripe - Citric: 2.2 Malic: 1.6 Succinic: 0.3	Yashoda *et al.,* 2006
AsA (mg g^{-1} FW)	According to Liu *et al.,* 2005	Ripe fruit (pulp)	Wacheng (W) Zihua (Z) (China)	Titrated with 2,6 dichlorophenolindophenol according to Liu *et al.,* 2005	0.25 (W), 0.27 (Z)	Zhao *et al*., 2006
Organic acids (citric acid) (mg g^{-1} DW)	AOAC, 1984, no further details	Ripe pulp	Ataulfo (Mexico)	5 g homogenised pulp samples were titrated with 0.1N NaOH using phenolphthalein as an indicator (AOAC, 1984)	29.1	Montalvo *et al*., 2007

AsA (mg g^{-1} FW)	5 g of fresh pulp samples were extracted with ultra pure water (15 ml) Vinci *et al*., 1995.	Ripe pulp	Haden (H) Tommy Atkins (TA) Palmer (P) Uba (U) (Brazil)	HPLC system	0.15 (H), 0.09 (TA), 0.1 (P) and 0.65 (U)	Ribeiro *et al*., 2007
AsA (mg g^{-1} FW)	1 g fresh pulp pulverised under LN and homogenized with 0.1% metamorphic acid (5 ml), homogenate was centrifuged for 20 min.	Ripe fruit pulp	Keitt (Brazil)	HPLC system using a µBondapak C18 column	0.25	Gomez *et al*., 2008
AsA (mg g^{-1} FW)	According to Doner and Hickts (1981). 10 g of pulp tissue homogenized with 50 ml of metaphosphoric acid-acetic acid.	Ripe fruit (pulp)	Ataulfo (A), Kent (Ke), Keitt (Ki) (Mexico)	HPLC system with NH_2 type µBondapak analytical column and UV detector	0.6(A), 0.06(Ke), 0.04(Ki)	Gonzalez-Aguilar *et al*., 2008
AsA (mg g^{-1} FW)	According to Liu *et al.,* 2005	Ripe fruit (pulp)	Tainong (China)	According to Liu *et al.,* 2005	0.38	Wang *et al*., 2009
Citric, oxalic, ascorbic, malic, tartaric and total acids (mg g^{-1} DW)	According to Thanaraj *et al*., 2009.	Ripe fruit (pulp)	Anwar Rotale (AR), Faiz Kareem (FK) and Chaunsa (C), (Venezuela)	According to Thanaraj *et al*., 2009	Citric acid: 16.7 (AR), 15.1 (FK), 8.7 (C) Malic acid: 6.23 (AR), 7 (FK), 9.4 (C) AsA: 3.52 (AR), 5.5 (FK), 0.9 (C) Oxalic acid: 1.04 (AR), 0.8 (FK), 0.8 (C) Tartaric acid: 3.31 (AR), 2.2 (FK), 2.7 (C) Total acids:30.8 (AR), 28 (FK), 22.7 (C)	Rajwana *et al*., 2010

HPLC: High performance liquid chromatography *LC: Liquid chromatography* *NaOH: Sodium hydroxide*
LN: Liquid nitrogen *AOAC: Association of official analytical chemist*

Appendix C 7.4 Total phenolics content of different mango cultivars extracted and analysed in different methods

Analytes	Extraction Method	Fruit maturity/ Section	Cultivar	Quantification Method	Range Measured	Reference
Total extractable Phenolics (mg GAE g^{-1} DW)	500 mg (mango peel) powder sample with 40ml MeOH: water (50:50,v/v) at room temp. for 60 min.	Fruit (peel)	Haden (Cuba)	Supernatant made up to 100 ml with distilled water and analysed spectrophotometically assayed by the Folin-Ciocalteau method using tannic acid as standard. Spectrophotometically at 700 nm.	44 – 70	Larrauri *et al.*, 1996
Total phenolics (mg GAE g^{-1} FW)	Extracted as per the Prussian Blue method as modified by Graham (1992). Samples were extracted with methanol (25 mlx3) after defatted with petroleum ether and chloroform. The extract was died and portioned between *n*-butanol:water to afford *n*-butanol soluble extract.	Raw and ripe (Peel and Pulp)	Deshi (De), Langra (L), Chausa (C), Mallika (M), Dashahari (Da) and Amprapali (A) (India)	Dried samples were re-suspended in 0.1 ml HPLC grade methanol, through vortexing and filtering, quantified with HPLC (UV-VIS detector).	Ripe - 4.1 (Da), 2.6 (De), 3.3 (M) and traces in other cultivars Unripe – compare to ripe relatively lower amount in all cultivars, 2.8 (M).	Singh *et al.,* 2004
Phenolic compounds (mg g^{-1} DW)	Extracted as per the method of Singh *et al.,* (2002). Fresh samples suspended in 5 ml ethanol water (80:20 v/v) then subjected to ultrasonication for 15 min at 4°C followed by centrifugation (12500xg) for 15 min.	Raw and ripe (Peel and Pulp)	Deshi (De), Langra (L), Chausa (C), Mallika (M), Dashahari (Da) and Amprapali (A) (India)	Analysed with Folin-Ciocalteau reagent. Expressed in Gallic acid equivalents	Tanic acid (2.72), gallic acid (2.86), ferulic acid (3.3), vanillic acid (3.14) and chlorogenic acid (4.16).	Singh *et al.,* 2004

Total Phenolics (µmol kg^{-1} FW)	No details	Peel and Pulp	Choke anan (Thailand)	Analysed using HPLC (Kondo *et al.,* 2002)	14 DAFB:477(peel), 313(pulp) 56 DAFB:1263(peel), 855(pulp) 84 DAFB:1142(peel), 802(pulp) 84 DAFB: peel: catechin (52)	Kondo *et al.,* 2005
Total extractable phenolics (mg GAE g^{-1} DW)	Extracted using aqueous organic solvents according to Jimenze-Escrig *et al.,* 2001.	Mature green preclimacteric mango (Peel and Pulp)	Tommy Atkins (Mexico)	Estimated by Folin-Ciocalteau method (Singleton *et al.,* 1999)	16.14	Vergara-Valencia *et al.,* 2007
TP (mg GAE g^{-1} FW)	According to Liu *et al*., 2005	Pulp ripe	Wacheng (China)	According to Liu *et al*., 2005	0.65	Zhao *et al*., 2006
Total Phenolics (mg GAE g^{-1} DW)	Homogenised with 80% EtOH or 80% acetone or 0.05M sodium phosphate buffer (pH 7.5), clear solution was obtained after centrifugation for 15 min at 10,000g.	Raw and Fruit (peel)	Raspuri (R) Badami (B) (India)	Estimated by Folin-Ciocalteau method (Jimenze-Escrig *et al.,* 2001) using gallic acid as standard.	Unripe (R): 29.4 (buffer extract) 73.88 (alcohol ext.) 109.7(acetone ext.) Unripe (B): 20.1 (buffer extract) 37.92(alcohol ext.) 90.18(acetone ext.) Ripe (R): 13.9 (buffer extract) 46.31(alcohol ext.) 100 (acetone ext.) Ripe (B): 9.84 (buffer extract) 33.31(alcohol ext.) 54.61(acetone ext.)	Ajila *et al.,* 2007a
TP (mg GAE g^{-1} FW)	Folin-Ciocalteau method (Swain and Hillis, 1959) used to extract from pulp juice.	Mature green Mid-ripe Ripe pulp	Tommy Atkins (USA)	Folin-Ciocalteau method (Swain and Hillis, 1959)	Mature green – 4 Mid-ripe – 3 Ripe – 0.17	Kim *et al*., 2007
TP (mg GAE g^{-1} FW)	1 g of fresh pulp extracted in 10 ml of MeOH:water (60:40)	Ripe pulp	Haden (H) Tommy Atkins (TA) Palmer (P) Uba (U) (Brazil)	Estimated calorimetrically by Folin-Ciocalteau method (Singleton *et al.,* 1999)	0.6 (H), 0.5 (TA), 1.3 (P) and 2.25 (U)	Ribeiro *et al*., 2007

TP (mg GAE g^{-1} DW)	Extracted according to Bloor (2001), 0.5 g peel dried power extracted in 20 ml of MeOH water (60/40).	Ripe peel	Uba (U) (Brazil)	Estimated calorimetrically by Folin-Ciocalteau method (Singleton *et al.,* 1999)	57.24 (U)	Ribeiro *et al*., 2008
TP (mg GAE g^{-1} DW)	According to Thanaraj *et al*., 2009. (Folin-Ciocalteau method)	Ripe fruit peel and pulp	Anwar Rotale (AR), Faiz Kareem (FK) and Chaunsa (C), (Venezuela)	According to Thanaraj *et al*., 2009. (Folin-Ciocalteau method)	Peel: 48.87 (AR), 29.8 (FK), 51.2 (C) Pulp: 1.89 (AR), 1.2 (FK), 1.2 (C)	Rajwana *et al*., 2010

GAE – Gallic acid equivalent
MeOH - Methanol

Appendix C 7.5 Flavonoids concentration of different mango cultivars extracted and analysed in different methods

Analytes	Extraction Method	Fruit maturity/ Section	Cultivar	Quantification Method	Range Measured	Reference
Flavonoids ($\mu g\ g^{-1}$ DW)	Dried peel samples (90°C for 1h and 30 min) was extracted using Rodriguez-Saona and Wrolstad (2001)	Fruit peel	Tommy Atkins (Brazil)	Separated using an Agilent HPLC system (1100) 4µ Synergi Hydro-RP with a DAD (G1315B)	**Dried mango peels** Mangiferin-1.7 Q 3 gal-0.7 Q 3 glc-0.6 Q 3 rhm-0.02 K 3 glc-0.04 Quercetin-0.07 Isomangiferin-0.13 Mangiferin gallate-0.32 Isomangiferin gallate-0.08 **Mango peel extract** Mangiferin-1.19 Q 3 glc-0.03 Quercetin-0.02 Isomangiferin-0.05 Mangiferin gallate-0.1 Isomangiferin gallate-0.02	Berardini *et al*., 2005
Flavonoids ($\mu g\ g^{-1}$, DW)	Extraction and purification according to Schieber *et al*., 2003	Fruit peel	Uba (Brazil)	Detected in Agilent HPLC series 1100 system with C18 Hydro Synergy column	Mangiferin-199 Q 3 gal-151 Q 3 glc-370 Q 3 rhm-15.8 K 3 glc-35.3 Quercetin-64.1	Ribeiro *et al*., 2008

Q 3 gal.: Quercetin 3 O-galactoside
Q 3 glc.: Quercetin 3 O-glucoside
Q 3 rhm.: Quercetin 3 O-rhamnoside
K 3 glc.: Kaemferol 3 O-glucoside
DAD: Diode array detector

Appendix C 7.6: Total carotenoids content of different mango cultivars extracted and analysed in different methods

Analytes	Extraction Method	Fruit maturity/ Section	Cultivar	Quantification Method	Range Measured	Reference
Total carotenoids (µg g^{-1} FW)	By using partition methodology (Association of Vitamin Chemists, 1953)	Ripe fruit pulp	Alphonso (India)	By using partition methodology (Association of Vitamin Chemists, 1953)	87.46	Padmini and Prabha, 1997
Total carotenoids (µg g^{-1} FW)	2 g of fresh pulp samples with 0.05 g magnesium carbonate was ground and extracted twice with a 20 ml of acetone-hexane (75:60) (Thomas, 1963)	Ripe fruit pulp	Kensington Pride (Australia)	UV/VIS spectrophotometer (6405)	67.13	Lalel *et al*., 2003b
Total carotenoids (µg g^{-1} DW)	5 g pulp powder in 15% KOH and extracted using Zakaria *et al.* (1979)	Ripe fruit pulp	Baneshan (B) Suvarnarekha (S) (India)	Spectrophotometer (Shimadzu)	39.47(B), 34.08(S)	Hymavathi and Khader, 2005
Total carotenoids (µg g^{-1} FW)	According to Bhaskar (1988)	Ripe fruit pulp	Kensington Pride (Australia)	UV/VIS spectrophotometer (6405)	21	Malik and Singh, 2006
Total carotenoids (µg g^{-1} FW)	1 g peel samples in 40 ml of MeOH containing 1 g KOH and extracted using Tee and Lim (1991)	Unripe and ripe fruit peel	Raspuri (R) Badami(B) (India)	Estimated using diffent colorimetric mtds, Davies (1976) method. Litchenthalar (1987) method	Unripe: 3945 (R), 1400(B) – Davis (1976) method. 3337(R), 1520(B)- Litchenthalar (1987) method Ripe:493 (R), 365(B) – Davis (1976) method. 547(R), 387(B)- Litchenthalar (1987) method	Ajila *et al*., 2007a

Total carotenoids (µg g^{-1} DW)	1 g peel samples in 40 ml of MeOH containing 1 g KOH and extracted using Tee and Lim (1991)	Unripe and ripe fruit peel	Raspuri (R) Badami(B) (India)	Estimated using different colorimetric methods, Davies (1976) method. Litchenthalar (1987) method	Unripe: 73.5 (R), 81(B) Ripe:436 (R), 194(B)	Ajila *et al*., 2007b
Total carotenoids (µg g^{-1} FW)	5 g of fresh pulp samples were extracted in distilled water and cold acetone and partitioned with petroleum ether	Ripe fruit pulp	Haden (H) Tommy Atkins (TA) Palmer (P) Uba (U) (Brazil)	According to Higby (1962)	19 (H), 25.5 (TA), 26.5 (P) and 24 (U)	Ribeiro *et al*., 2007
Total carotenoids (µg g^{-1} FW)	According to Lalel *et al.* (2003a) as b-carotene equivalent.	Ripe fruit pulp	Sindhri (S) Chaunsa (C) (Pakistan)	According to Lalel *et al.,* (2003) as b-carotene equivalent	76.6(S), 73.4(C)	Maqbool and Malik (2008)

UV/VIS: Ultraviolet visible spectroscopy
KOH: Potassium hydroxide
MeOH: Methanol

Appendix C 7.7: Volatile concentration of different mango cultivars extracted and analysed in different methods

Analytes	Extraction Method	Fruit maturity/ Section	Cultivar	Quantification Method	Range Measured	Reference
Volatiles (ppb FW)	20 g fresh fruit pulp in 50 ml sat. NaCl and 7 ml solution extracted at 60°C for 30 min. using a HS-SPME.	Fresh fruit pulp	Alphonso (A) Baladi (B) (Egypt)	Hewlett Packard GC-FID (5890) coupled with DB 5MS capillary column.	Toluene-50(A), 40(B), a-pinene-900(A), 800(B), b-pinene-200(A), 50(B), myrcene-19000(A), 17000(B), limonene-300(A), 40000(B), ocimene-7500(A), 5000(B), a-terpinolene-5(A), 40(B), a-humulene-800(A), 1300(B), a-caryophyllene-1400(A), 2300(B)	Engel and Tressl, 1983
Volatiles (µg kg^{-1} FW)	Linkens and Nickerson apparatus (distillation extraction with 15 ml pentane as solvent)	Fresh ripe pulp	Willard (W) Karutha Colomban (KC) Parrot (P) (Sri Lanka)	GE-EISM and GC-CIMS along with GC-MS AND GC-FID (fused silica capillary column-PEG 20M or OV 101 OR BP20 bonded phase)	tolune-41.1(W), 6.8(KC),22.6(P) a-pinene-27.9(W), 0(KC),10(P) b-pinene-8.9(W), 7.3(KC),17(P) myrcene-5.9(W), 10.8(KC),13.8(P) 3carene-11.4(W), 0(KC),73.5(P) a-terpinene-5.9(W), 0(KC),24.5(P) limonene-16(W), 6.5(KC),19.5(P) ocimene-4.2(W), 95.1(KC),11.9(P) a-terpinolene-135.5(W), 0(KC),219.8(P) a-caryophyllene-10.1(W), 11.5(KC),6.3(P) a-humulene-5.9(W), 6.5(KC),7.5(P)	MacLeod and Pieris, 1984
Volatiles (not quantified)	HS-SPME, 3 ml fresh fruit slurry with 1.1 ml NaCl in auto sample at 4°C	Fresh pulp slurry	Keitt Palmer (USA)	GC-MS	a-pinene, myrcene, 3carene, limonene, b-pinene, a-terpinene, a-terpinolene, a-humulene (identified)	Beaulieu and Lea, 2003
Volatiles (µg kg^{-1} FW)	HS-SPME	Fresh fruit pulp	Kensington pride (Australia)	Hewlett Packard GC-FID (5890) II couples with Hewlett Packard mass selective detector (5791)	a-pinene-197, myrcene-187.8, 3carene-1074.7, a-terpinolene-5771.2, a-humulene-456.7, a-caryophyllene-869.1	Lalel *et al*., 2003a

Volatiles (µg kg^{-1} FW)	20 g fresh fruit pulp in 50 ml sat. NaCl and 7 ml solution extracted at 60°C for 30 min. using a HS-SPME.	Fresh fruit pulp	Kensington pride (Australia)	Hewlett Packard GC-FID (5890) coupled with DB 5MS capillary column.	a-pinene-21.8, b-pinene-1.65, myrcene-64.4, 3carene-256, a-terpinene-30.7, limonene-38, ocimene-6.7, a-terpinolene-2815	Lalel *et al*., 2003b
Volatiles (µg kg^{-1} FW)	HS-SPME	Fresh fruit pulp	Delta R2E2 (Australia)	Hewlett Packard GC-FID (5890) coupled with DB 5MS capillary column and Hewlett Packard mass selective detector (5971) (Lalel *et al*., 2003b)	a-pinene-19.31, b-pinene-1.28, myrcene-19.25, 3carene-117.3, a-terpinene-10.71, limonene-15.56, ocimene-3.74, a-terpinolene-944.12	Lalel and Singh, 2006
Volatiles (µg kg^{-1} FW)	200 g of fresh pulp in 400 ml of water and methyl nonanoate (0.2 mg) as IS and extracted using simultaneous distillation-extraction (SDE) technique with 30 ml pentane: diethyl ether (1:1) as solvent for 1 h.	Fresh fruit pulp	Hilacha (Hi) Haden (Ha) Irwin (I) Manila (M) Springfield (S) Tommy Atkins (TA) Van Dyke (VD) Vallenato (V) Yulima (Y) (Colombia)	GC-FID and GC-MS	a-pinene-8200(Hi), 600(Ha),700(I), 0(M), 300(S), 400(TA), 1900(VD), 5100(V), 700(Y) b-pinene-2400(Hi), 100(Ha), 500(I), 0(M), 400(S), 400(TA), 200(VD), 500(V), 200(Y) myrcene-2700(Hi), 100(Ha), 400(I), 400(M), 400(S), 400(TA), 400(VD), 900(V), 100(Y) 3carene-100(Hi), 48100(Ha), 7200(I), 15100(M), 400(S), 10100(TA), 100(VD), 400(V), 0(Y) a-terpinene-200(Hi), 400(Ha), 0(I), 0(M), 0(S), 100(TA), 0(VD), 400(V), 400(Y) limonene-3800(Hi), 2900(Ha), 1100(I), 1500(M), 800(S), 100(TA), 600(VD), 1700(V), 400(Y) ocimene-100(Hi), 100(Ha), 600(I), 600(M), 500(S), 0(TA), 100(VD), 100(V), 100(Y) a-terpinolene-400(Hi), 3100(Ha), 0(I), 1100(M), 500(S), 600(TA), 800(VD), 1900(V), 13100(Y)	Quijano *et al*., 2007
Volatiles (µg kg^{-1} FW)	Combined simultaneous distillation-extraction (SDE) technique with pentane as solvent (Godefroot *et al*., 1981)	Fresh pulp	Kent (Spain)	GC-MS Finnigan TRACE MS chromatograph with fused silica capillary column DB5.	a-pinene-10, myrcene-17, 3carene-401, limonene-13	Torres *et al.,* 2007

Volatiles ($\mu g\ kg^{-1}$ FW)	10 g fresh fruit pulp in 35 ml sat. NaCl and 7 ml solution extracted at 50°C for 30 min. using a HS-SPME.	Fresh pulp	Kensington pride (Australia)	GC-FID (Agilent), capillary column HP-5 MS agilent,	a-pinene-27.5, myrcene-32.4, 3carene-107, limonene-23.3, b-pinene-1.5, a-terpinene-47, a-terpinolene-966.7, a-humulene-31,ocimene-0.97, a-caryophyllene-47.9	Dang *et al*., 2008a
Volatiles ($\mu g\ kg^{-1}$ FW) (after 3 weeks cold storage at 13°C)	-do-	-do-	-do-	-do-	a-pinene-55.9, myrcene-64, 3carene-169.4, limonene-56.5, b-pinene-2.6, a-terpinene-108, a-terpinolene-2517.2, a-humulene-33.9,ocimene-2, a-caryophyllene-52.5	Dang *et al*., 2008b
Volatiles (not quantified)	2 g of fresh mango pulp in 20 ml distilled water. Helium swept through homogenate (20 ml/min) and trapped on activated charcoal and graphite for 1 h at 37°C. Desorption was by MW-1 microwave sampler (Rektorik, 1985).	Fresh fruit pulp	Cogshall (Franch)	3400 GC-FID using a DB-Wax column.	tolune, a-pinene, b-pinene, myrcene, 3carene, a-terpinene, limonene, ocimene, a-terpinolene, b-caryophyllene, a-humulene (identified with major of 3carene)	Lebrun *et al*., 2008
Volatiles (not quantified)	Diluted 50% with deionised water (2 ml) fast frozen in LN and equilibrated at 80°C for 15 min in a static HS sampler.	Fresh fruit pulp	Keitt Kent (Florida, USA)	Perkin-Elmer 8500 GC-FID - a DB WAX column with polar coating	a-pinene, b-pinene, myrcene, 3carene, a-terpinene, limonene, b-caryophyllene, (identified with major of 3carene)	Lebrun *et al*., 2008
Volatiles ($\mu g\ g^{-1}$ FW)	10 g of fresh pulp grounded in LN and extracted for 1 h at 28°C using 40 ml of dichloromethane and nonyle acetate (40 µg) as IS. Supernatant was dehydrated with sodium sulphate and then concentrated.	Fresh fruit pulp	Alphonso (1) Badami (2) Chandrama (3) Chittur Badami (4) Dasheri (5) Dudh peda (6) Goamankur (7) Gopta of Navasari	Clarus 500 Perkin-Elmer GC-FID with Rtx-5 MS capillary Column.	a-pinene-3.98(1), 7.44(2),0.16(3), 4.4(4), 1.95(5), 5.41(6), 0.28(7), 1.92(8), 0.54(9), 0.46(10), 25.18(11), 25.21(12), 0.67(13), 4.8(14), 7.03(15), 136.6(16), 0.5(17), 6.76(18), 4.25(19), 1.14(20), 21.49(21), 12.18(22), 0.13(23), 0.1(24), 2.02(25), 14.13(26), 3.21(27) b-pinene-1.17(1), 0.22(2), 0.12(3), 0.29(4), 0.1(5), 3.69(6), 0.17(7),	Pandit *et al*., 2009a and b

	(8) Keitt (9), Kent(10), Kesar (11), Langra (12), Lili (13), Makaram(14), Maya(15), Mulgoba(16), Musharad(17), Neelum(18), Osteen(19), Pairi(20), Rajapuri(21), Ratna(22), Sabja(23), SB Chausa(24), Sindhu(25), Totapuri(26), Villai Kolumban(27) (India)	3.11(8), 0.85(9), ND(10), 0.72(11), 0.51(12), 0.1(13), 1.58(14), 0.75(15), 13.15(16), 0.54(17), 0.56(18), 3.42(19), ND(20), 1.85(21), 1.38(22), ND(23), ND(24), 0.48(25), 1.64(26), 0.27(27) myrcene-8.93(1), 0.78(2),2.01(3), 140.2(4), 4.44(5), 852.5(6), 0.62(7), 1.61(8), 0.79(9), 1.28(10), 12.52(11), 67.67(12), 2.46(13), 2.67(14), 5.1(15), 680.3(16), 180(17), 30.35(18), 8.28(19), 14.96(20), 121.2(21), 3.16(22), 50.19(23), 0.11(24), 0.69(25), 102.9(26), 8.61(27) 3carene-0.05(1), ND(2),ND(3), 15.79(4), ND(5), 13.86(6), ND(7), ND(8), 4.92(9), 17.97(10), ND(11), 2046(12), 89.7(13), 0.53(14), 163.9(15), 4.18(16), ND(17), 5.13(18), 239.7(19), 4.86(20), 13.6(21), 2.23(22), 0.27(23), 1.3(24), 0.15(25), ND(26), 23.67(27) b-terpinene-ND(1), ND(2),ND(3), 0.88(4), ND(5), ND(6), ND(7), ND(8), ND(9), 1.19(10), ND(11), 2.47(12), 0.16(13), 0.37(14), 0.1(15), 1.79(16), ND(17), 0.1(18), 0.98(19), ND(20), 0.83(21), 0.33(22), ND(23), 0.02(24), ND(25), 0.13(26), 0.71(27) limonene-0.59(1), ND(2),0.28(3), 3.61(4), 311.4(5), 5.28(6), 0.27(7), 2.44(8), 1.41(9), 1.25(10), 0.55(11), 74.23(12), 3.52(13), 1.76(14), 6.56(15), 4.84(16), ND(17), 1.38(18), 15.39(19), 1.04(20), 3.41(21), 1.5(22), 0.4(23), 0.24(24), 0.52(25), 0.67(26), 11.87(27) ocimene-1055(1), 3.44(2),4.73(3), 1.31(4), ND(5), 93.05(6), 7.4(7), 2.27(8), 0.86(9), 1.58(10), 598.3(11), 14.85(12), 35.54(13), 9.21(14), 1.57(15), 89.2(16), 27.75(17), 0.27(18), 1.72(19), 6.93(20), 13.97(21), 52.61(22), 1.94(23), 0.07(24), 19.55(25), 58.93(26), 1.33(27) a-terpinolene- 0.18(24), ND in all others

			humulene-13.04(1), 5.13(2),0.32(3), 10.1(4), 8.07(5), 12.32(6), 3.33(7), 24.11(8), 1.01(9), 4.29(10), 8.7(11), 21.57(12), 1.87(13), ND(14), 2.01 (15), 11.61 (16), ND(17), 1.67(18), 4.55(19), 8.11(20), 3.87(21), 2.29(22), 34.06(23), 1.33(24), 2.3(25), 3.02(26), 1.22(27) a-caryophyllene-23.89(1), 10.77(2),1.47(3), 19.99(4), 15.67(5), 21.8(6), 6.63(7), 49.44(8), 1.8(9), 8.44(10), 16.82(11), 43(12), 3.53(13), 5.13(14), 4.64(15), 23.43(16), 12.62(17), 3.1(18), 9.68(19), 14.2(20), 7.95(21), 4.75(22), 70.80(23), 2.04(24), 4.42(25), 6.36(26), 2.71(27)

GC-EIMS: Gas chromatography - Electron ionization mass spectrometry
GC-CIMS: Gas chromatography - Chemical ionization mass spectrometry
GC-FID: Gas chromatography – flame ionization detector
HS-SPME: Headspace – solid phase micro extraction
GC-MS: Gas chromatography – mass spectrometry

Appendix D

D. 1: Sugar concentrations at vertical sections of pre-climacteric Sri Lankan mango fruits

Cultivar	Vertical Section	Fructose (mg g^{-1}) DW	Glucose (mg g^{-1}) DW	Sucrose (mg g^{-1}) DW	Total Sugar (mg g^{-1}) DW
	Stem end	84.20	65.60	51.28	201.10
Willard	Middle	88.60	71.00	48.85	208.40
	Distal end	87.00	68.80	50.91	206.60
	Stem end	64.50	22.20	23.32	110.10
Karutha Colomban	Middle	63.50	23.20	29.00	115.70
	Distal end	63.10	25.60	27.07	115.80
	Stem end	131.10	91.60	50.85	273.50
Malgova	Middle	127.80	84.30	46.76	258.80
	Distal end	129.90	74.90	41.31	246.10
	Stem end	93.70	58.70	23.80	176.20
Ampalavi	Middle	95.50	60.10	23.53	179.10
	Distal end	115.40	72.90	26.87	215.20
	Stem end	64.90	20.70	17.82	103.50
Vellai Colomban	Middle	64.20	17.20	22.74	104.20
	Distal end	63.80	17.90	18.94	100.70
LSD *(P = 0.05)*		17.46	15.99	9.24	35.35

D.2: LSD values of tested parameters of pre-climacteric Sri Lankan mango cultivars. Reference to tables 3.2 and 3.3 of chapter 3.

Tested Parameters	Starch (DW)	TP (DW)	Sugars (mg g^{-1} DW)				Organic acids (mg g^{-1} DW)				
			Fructose	Glucose	Sucrose	Total Sugars	Citric acid	Malic acid	Oxalic acid	Tartaric acid	Total acids
Cultivars	2.88	4.49	10.08	9.23	5.34	20.35	8.91	4.73	0.33	0.72	10.69
Maturity	2.88	3.48	7.81	7.15	4.13	15.76	6.90	3.67	0.26	0.56	8.28
HT (peel and pulp)	2.88	3.48	7.81	7.15	4.13	15.76	6.90	3.67	0.26	0.56	8.28
Cultivars x Maturity	4.98	7.78	17.46	15.99	9.24	35.25	15.43	8.20	0.58	1.25	18.51
Cultivars x HT	4.98	7.78	17.46	15.99	9.24	35.25	15.43	8.20	0.58	1.25	18.51
Maturity x HT	4.98	6.03	13.52	12.38	7.16	27.30	11.95	6.35	0.45	0.97	14.34
Cultivars x HT x Maturity	8.63	13.47	30.24	27.69	16.01	61.05	26.72	14.20	1.00	2.16	32.06

HT – Horizontal Transection

D.3: Biochemical composition (mg g^{-1}) of pre-climacteric Sri Lankan mango fruit of cv. Willard. Reference to figure 3.1(a)

Sample	Samples ID (PCA bi-plot)	DW-Fruc	DW-glu	DW-suc	DW-Tot.sug	DW-OA	DW-TA	DW-MA	DW-AA	DW-CA	DW-Tot.acids	DW-Tot.P	DW-starch
Peel	1C1	29.018	37.65502	59.89118	126.5642	0.184008	0	2.721939	10.9089	0.294705	14.10955	16.3	15.71646
	4C1	26.56543	45.62346	63.05979	135.2487	0.487537	0	0.029038	26.55932	9.133416	36.20932	13.7	20.23449
	7C1	22.72597	39.0662	59.09839	120.8906	0.614258	0.498245	0.34929	33.03677	15.71682	50.21538	15.75	15.65014
	10C1	22.2681	41.85752	61.64076	125.7664	0.301593	0.179336	5.997755	13.89168	1.481933	21.8523	14.5	21.07482
	13C1	18.94638	39.56647	58.36265	116.8755	0.132631	0.295377	6.694326	28.23221	11.73609	47.09064	13.9	20.26705
	16C1	25.06151	46.84782	66.9803	138.8896	0.22518	0.23127	4.943555	34.53345	18.25687	58.19032	15.35	13.62055
	19C1	47.7122	39.22512	69.3144	156.2517	0.220542	0.384639	0.045834	15.146	0.168752	15.96577	13.15	15.27982
	22C1	42.97468	74.04803	95.37989	212.4026	0.135679	0.05518	0.112215	30.00717	7.977939	38.28818	14.85	20.42617
	25C1	32.63351	46.83793	76.47508	155.9465	0.163122	0.438521	0.375271	36.92119	15.52632	53.42442	20.95	16.18536
Pulp	2C1	58.23838	72.92055	93.83918	224.9981	0.487537	0	0.029038	26.55932	9.133416	36.20932	13.7	20.23449
	3C1	75.92066	70.88914	87.15619	233.966	0.614258	0.498245	0.34929	33.03677	15.71682	50.21538	15.75	15.65014
	5C1	60.62206	70.52089	86.11579	217.2587	0.301593	0.179336	5.997755	13.89168	1.481933	21.8523	14.5	21.07482
	6C1	75.61964	64.23676	83.11144	222.9678	0.132631	0.295377	6.694326	28.23221	11.73609	47.09064	13.9	20.26705
	8C1	56.19608	70.14903	85.45202	211.7971	0.22518	0.23127	4.943555	34.53345	18.25687	58.19032	15.35	13.62055
	9C1	78.75514	62.79107	80.23312	221.7793	0.220542	0.384639	0.045834	15.146	0.168752	15.96577	13.15	15.27982
	11C1	63.23583	66.80075	78.26161	208.2982	0.135679	0.05518	0.112215	30.00717	7.977939	38.28818	14.85	20.42617
	12C1	77.00854	72.31974	82.87123	232.1995	0.163122	0.438521	0.375271	36.92119	15.52632	53.42442	20.95	16.18536
	14C1	70.55232	78.50011	93.29869	242.3511	0.130448	0.312298	0.249287	9.426155	1.078731	11.19692	10.95	18.48872
	15C1	52.01283	82.65777	96.86245	231.5331	0.158409	0.402289	0.077444	18.81177	8.066852	27.51676	14.3	23.84609
	17C1	67.24896	81.0987	91.66985	240.0175	0.052126	0.367085	0.197562	21.02026	10.82703	32.46406	16.3	23.81651
	18C1	79.99699	80.17876	98.36827	258.544	0.126481	0.499853	0.204061	9.274455	0.258157	10.36301	17.65	18.48211
	20C1	40.66182	87.67885	107.9534	236.294	0.041322	0.486566	0.008173	18.36182	7.565156	26.46304	17.95	25.93073
	21C1	47.49712	100.9463	117.1437	265.5871	0.246812	0.323228	0.14969	15.67678	9.805157	26.20166	18.45	19.37407
	23C1	43.644	101.9955	115.9367	261.5762	0.111187	0.316703	0.158066	8.900714	0.29129	9.77796	16.05	15.42049
	24C1	48.71034	81.68172	104.9717	235.3638	0.151142	0.487771	0.084552	18.77904	4.656997	24.1595	17.7	21.66335
	26C1	52.86055	97.69407	110.4343	260.989	0.109606	0.349132	0.042673	14.58302	8.117026	23.20146	16.75	27.27795
	27C1	42.69992	94.17835	114.0241	250.9024	0.0999	0.442325	0.061379	6.771329	0.290223	7.665156	20.6	20.90514

D.4: Comparison of 62.5% methanol and 80% ethanol extraction of sugars using ELSD detector with Dionex HPLC system

Sample	62.5% methanol extraction (mg g^{-1})				80% ethanol extraction (mg g^{-1})			
	Fructose (DW)	Glucose (DW)	Sucrose (DW)	Total Sugars (DW)	Fructose (DW)	Glucose (DW)	Sucrose (DW)	Total Sugars (DW)
Willard peel 1	69.54	47.57	142.92	260.03	72.51	48.91	150.32	271.73
Willard peel 2	65.09	40.79	114.67	220.54	71.78	45.84	131.30	248.91
Willard peel 3	70.23	48.98	129.20	248.41	52.53	37.74	102.95	193.23
Willard pulp 1	117.06	80.18	385.11	582.35	106.32	71.34	355.93	533.60
Willard pulp 2	110.37	74.38	387.25	572.01	118.05	80.24	409.19	607.48
Willard pulp 3	104.45	75.28	405.32	585.05	92.93	67.36	349.43	509.72

D.5: LSD values of tested parameters for the methanol and ethanol extraction of sugars (mg g^{-1})

Tested Parameters	Fructose	Glucose	Sucrose	Total Sugars	F value
Sample type (peel and pulp)	12.16	6.84	29.52	45.58	< 0.001
Extraction method	12.16	6.84	29.52	45.58	0.40 – 0.42
Sample type x extraction method	17.20	9.68	41.74	64.46	0.45 – 0.84

D.6: Comparison of RID detector with Agilent HPLC system and ELSD detector with Dionex HPLC system in the analysis of sugars extracted using 62.5% methanol

Sample	RID detector with Agilent HPLC system (mg g^{-1})				ELSD detector with Dionex HPLC system (mg g^{-1})			
	Fructose (DW)	Glucose (DW)	Sucrose (DW)	Total Sugars (DW)	Fructose (DW)	Glucose (DW)	Sucrose (DW)	Total Sugars (DW)
Willard peel 1	93.26	72.49	139.42	305.17	69.54	47.57	142.92	260.03
Willard peel 2	93.65	61.58	115.18	270.41	65.09	40.79	114.67	220.54
Willard peel 3	98.19	71.33	135.38	304.91	70.23	48.98	129.20	248.41
Willard pulp 1	125.46	105.20	310.16	540.82	117.06	80.18	385.11	582.35
Willard pulp 2	139.29	92.28	361.69	593.27	110.37	74.38	387.25	572.01
Willard pulp 3	129.41	94.19	366.74	590.35	104.45	75.28	405.32	585.05

D.7: LSD values of tested parameters for the methanol extraction and analysis of sugars (mg g^{-1}) using RID and ELSD detectors

Tested Parameters	Fructose	Glucose	Sucrose	Total Sugars	F value
Sample type (peel and pulp)	6.85	2.17	25.54	27.67	<0.001
Analysis (RID and ELSD)	6.85	2.17	25.54	27.67	<0.001
Sample type x Analysis	9.69	3.07	36.12	39.13	0.05 – 0.74

D.8: Concentration of flavonoids of mango cv. Karutha Colomban (µg g^{-1})

Trt	DW-Mangi	DW-Q 3-O-gala	DW-Q 3-O glc	DW-Q 3-O-rham	DW-K 3-O-glc	DW-Quercetin
0-control	4194.947	81.98215	265.6448	21.3589	10.65076	67.34158
3KP-20-30	5172.565	268.6147	305.3689	55.97487	38.86949	49.05041
6KP-30-30	2872.026	89.82805	288.1951	57.63871	53.91868	37.92326
6KP-20-30	3361.798	153.7051	394.9009	71.19011	79.1481	41.23517
6KP-20-20	4539.565	122.7811	339.0792	96.82096	50.99042	64.88102

D.9 : Concentration of flavonoids of mango cv. Willard (μg g^{-1})

Trt	DW-Mangi	DW-Q 3-O-gala	DW-Q 3-O glc	DW-Q 3-O-rham	DW-K 3-O-glc	DW-Quercetin
0-control	2955.698	555.6346	350.1961	25.44393	11.83658	32.42644
20-30-3	4282.732	479.0089	359.5415	36.18844	48.53549	41.77271
30-20-3	7151.742	494.28	450.1079	63.77906	40.2991	63.67274
20-20-6	6113.918	612.1456	530.7021	64.49301	44.7521	75.33543
30-30-6	5658.811	393.9302	333.593	38.99312	33.7647	39.54385
20-30-6	7633.082	447.1243	398.251	28.51274	29.47098	57.87867
30-20-6	4406.857	471.7953	335.6159	72.75841	26.9061	43.67462

D.10: Two-sided test of correlations different among biochemical compounds of mango cvs. Willard, Karutha Colomban and Malgova peel ripened at 32^{o}C from zero Probabilities

	AsA	Citric_Acid	Flv_K_glc	Flv_Q_gal	Flv_Q_glc	Flv_Q_rhm	Flv_Qerc	Flv_mangi	Fructose	Glucose	Malic_Acid	Oxalic_Acid	Sucrose	TP	T_OA	T_Sugars	Tar
AsA	1																
Citric_Acid	-0.121	1															
Flv_K_glc	-0.149	-0.243	1														
Flv_Q_gal	0.951	-0.167	-0.057	1													
Flv_Q_glc	0.863	-0.243	0.34	0.904	1												
Flv_Q_rhm	-0.806	0.056	-0.07	-0.624	-0.716	1											
Flv_Qerc	0.252	-0.464	0.791	0.347	0.635	-0.292	1										
Flv_mangi	0.501	0.073	0.057	0.681	0.576	-0.033	0.35	1									
Fructose	-0.714	0.096	-0.392	-0.597	-0.804	0.917	-0.586	-0.117	1								
Glucose	0.563	0.707	-0.146	0.467	0.434	-0.583	-0.127	0.276	-0.543	1							
Malic_Acid	-0.885	-0.124	0.405	-0.771	-0.586	0.733	0.082	-0.42	0.517	-0.627	1						
Oxalic_Acid	-0.234	0.987	-0.21	-0.284	-0.342	0.139	-0.483	0.032	0.176	0.62	-0.045	1					
Sucrose	-0.026	-0.344	0.021	0.018	0.01	0.046	-0.257	-0.34	0.151	-0.248	0.115	-0.361	1				
TP	0.617	0.167	0.468	0.629	0.816	-0.63	0.664	0.499	-0.833	0.649	-0.382	0.083	-0.39	1			
T_OA	0.616	0.685	-0.296	0.581	0.445	-0.453	-0.18	0.486	-0.39	0.952	-0.686	0.589	-0.269	0.586	1		
T_Sugars	-0.269	0.05	-0.294	-0.194	-0.349	0.411	-0.645	-0.253	0.568	-0.174	0.191	0.055	0.836	-0.618	-0.111	1	
Tartaric_acid	0.826	-0.103	-0.329	0.858	0.652	-0.403	0.023	0.619	-0.337	0.44	-0.733	-0.195	0.032	0.382	0.596	-0.023	

D.11: Two-sided test of correlations different among biochemical compounds of mango cvs. Willard, Karutha Colomban and Malgova pulp ripened at 32°C from zero Probabilities

1															
Citric_Acid	-0.22	1													
Fructose	0.347	-0.26	1												
Glucose	0.954	-0.072	0.111	1											
Malic_Acid	-0.409	0.414	-0.023	-0.453	1										
Oxalic_Acid	-0.265	0.837	-0.009	-0.219	0.595	1									
Sucrose	-0.857	-0.177	-0.494	-0.825	0.165	-0.1	1								
Sugar_acid	0.064	-0.747	0.357	-0.107	0.233	-0.45	0.122	1							
TP	0.832	-0.081	0.071	0.865	-0.338	-0.113	-0.638	-0.081	1						
TSS	0.273	-0.256	-0.231	0.248	0.259	-0.435	-0.077	0.389	0.175	1					
TTA	-0.202	0.881	-0.443	-0.033	0.153	0.592	-0.059	-0.885	-0.125	-0.139	1				
T_OA	-0.131	0.994	-0.206	0.004	0.421	0.851	-0.262	-0.727	-0.003	-0.231	0.861	1			
T_Sugars	-0.663	-0.52	-0.065	-0.749	0.009	-0.297	0.872	0.396	-0.538	-0.154	-0.429	-0.582	1		
T_carods	-0.001	-0.753	0.345	-0.168	0.153	-0.577	0.166	0.923	-0.271	0.462	-0.758	-0.749	0.427	1	
Tartaric_acid	0.499	-0.445	0.657	0.269	-0.115	-0.128	-0.305	0.324	0.453	0.033	-0.455	-0.37	0.099	0.232	1
	AsA	Citric_Acid	Fructose	Glucose	Malic_Acid	Oxalic_Acid	Sucrose	Sugar_acid	TP	TSS	TTA	T_OA	T_Sugars	T_carods	Tartaric_acid

D. 12(a): Correlation matrix for sugars, organic acids, TP and startch of pre-climacteric mango cv. Willard

AA	1.000											
CA	0.879	1.000										
MA	0.308	0.237	1.000									
OA	0.497	0.380	0.154	1.000								
Starch_%	-0.350	-0.208	-0.122	-0.371	1.000							
TA	-0.117	-0.055	-0.247	-0.079	-0.448	1.000						
TP	0.065	0.152	-0.142	-0.074	0.261	0.075	1.000					
T_acids	0.980	0.938	0.402	0.467	-0.316	-0.112	0.078	1.000				
T_sug	0.254	0.530	-0.189	-0.079	0.444	-0.152	0.166	0.322	1.000			
fruct	-0.063	0.227	-0.256	-0.179	0.521	-0.190	0.088	0.006	0.917	1.000		
gluc	0.033	0.303	-0.226	-0.165	0.533	-0.230	0.063	0.097	0.945	0.973	1.000	
sucr	0.685	0.839	-0.006	0.140	0.092	0.031	0.280	0.729	0.722	0.398	0.468	1.000
	AA	CA	MA	OAS	starch_%	TA	TP	T_acids	T_sug	fruct	gluc	sucr

D. 12(b): Correlation matrix for sugars and organic acids of cvs. Willard, Karuhtha Colomban, Malgova, Vellai Colamban and Ampalavi

AA	1									
CA	0.354	1								
MA	0.296	0.045	1							
OA	0.251	0.112	0.154	1						
TA	0.339	0.274	0	0.04	1					
T_acids	0.846	0.795	0.272	0.23	0.382	1				
fruct	0.45	-0.045	0.01	-0.23	0.044	0.255	1			
gluc	0.705	-0.017	0.113	-0.101	0.247	0.439	0.764	1		
sucr	0.435	0.352	0.129	-0.047	0.071	0.479	0.423	0.324	1	
T_sug	0.655	0.076	0.096	-0.159	0.158	0.459	0.913	0.897	0.628	1
	AA	CA	MA	OA	TA	T_acids	fruct	gluc	sucr	T_sug

D. 13(a): Correlation matrix for biochemical compounds of cv. Willard pulp ripened at 20°C and 30°C

DW_AsA	1.000											
DW_CA	0.426	1.000										
DW_MA	-0.072	0.016	1.000									
DW_OA	0.383	0.910	-0.204	1.000								
DW_TA	0.356	-0.125	-0.258	-0.295	1.000							
DW_T_acids	0.469	0.975	0.199	0.820	-0.053	1.000						
DW_Tot_sugars	-0.525	-0.707	-0.406	-0.665	0.087	-0.782	1.000					
DW_fructose	-0.737	-0.370	-0.276	-0.420	0.192	-0.420	0.734	1.000				
DW_glucose	0.443	0.898	0.166	0.884	-0.161	0.905	-0.924	0.568	1.000			
DW_sucrose	-0.288	-0.809	-0.300	-0.740	0.047	-0.856	0.908	0.398	-0.949	1.000		
TP	0.780	0.352	-0.039	0.523	-0.223	0.332	-0.552	-0.867	0.486	-0.265	1.000	
T_Caro	-0.353	-0.818	-0.308	-0.667	-0.123	-0.887	0.881	0.376	-0.909	0.970	-0.182	1.000
	AsA	CA	MA	OA	TA	T_acids	Tot_sugars	fructose	glucose	sucrose	TP	T_Caro

D. 13(b): Correlation matrix for biochemical compounds of cv. Karutha Colomban pulp ripened at 20°C and 30°C

DW_AsA	1.000											
DW_CA	0.990	1.000										
DW_MA	-0.435	-0.504	1.000									
DW_OA	-0.523	-0.593	0.972	1.000								
DW_TA	0.947	0.941	-0.191	-0.311	1.000							
DW_T_acids	0.992	1.000	-0.478	-0.569	0.951	1.000						
DW_Tot_sugars	-0.751	-0.670	-0.236	-0.147	-0.821	-0.690	1.000					
DW_fructose	-0.442	-0.316	-0.352	-0.277	-0.437	-0.333	0.832	1.000				
DW_glucose	0.744	0.821	-0.736	-0.846	0.683	0.809	-0.175	0.167	1.000			
DW_sucrose	-0.929	-0.939	0.232	0.337	-0.990	-0.947	0.756	0.315	-0.716	1.000		
TP	0.691	0.695	-0.810	-0.743	0.446	0.679	-0.245	-0.100	0.562	-0.459	1.000	
T_Caro	-0.961	-0.983	0.649	0.730	-0.867	-0.977	0.540	0.218	-0.882	0.870	-0.767	1.000
	DW_AsA	DW_CA	DW_MA	DW_OA	DW_TA	T_acids	Tot_sugars	fructose	glucose	sucrose	TP	T_Caro

D. 13(c): Correlation matrix for biochemical compounds of cv. Willard peel ripened at 20°C and 30°C

%_wt_loss	1.000						
DW_AsA	-0.526	1.000					
DW_CA	0.025	-0.213	1.000				
DW_K_3_O_glc	0.164	0.068	-0.146	1.000			
DW_MA	0.067	0.427	-0.549	0.313	1.000		
DW_Mangi	0.629	0.013	-0.555	0.430	0.746	1.000	
DW_OA	-0.845	0.239	0.460	-0.355	-0.484	-0.926	1.000
DW_Q_3_O_gala	-0.676	0.133	-0.440	0.015	0.272	-0.219	0.385
DW_Q_3_O_glc	-0.074	0.083	-0.773	0.496	0.705	0.536	-0.379
DW_Q_3_O_rham	0.269	-0.183	0.086	0.359	0.346	0.194	-0.233
DW_Quercetin	0.206	-0.017	-0.665	0.560	0.805	0.746	-0.604
DW_TA	-0.054	0.690	-0.536	0.108	0.705	0.418	-0.283
DW_T_acids	-0.160	0.725	0.183	0.095	0.540	0.116	0.081
DW_Tot_sugars	0.284	0.469	-0.617	0.337	0.747	0.705	-0.627
DW_fructose	0.245	0.160	0.005	-0.536	-0.357	-0.125	-0.083
DW_glucose	-0.864	0.769	-0.357	-0.233	0.206	-0.295	0.549
DW_sucrose	0.825	-0.545	0.057	0.524	0.247	0.608	-0.738
TP	0.341	-0.667	-0.061	-0.148	0.216	0.207	-0.278
TSS%	0.794	-0.433	-0.059	-0.319	-0.164	0.411	-0.634
	%_wt_loss	DW_AsA	DW_CA	DW_K_3_O_glc	DW_MA	DW_Mangi	DW_OA

DW_Q_3_O_gala	1.000											
DW_Q_3_O_glc	0.674	1.000										
DW_Q_3_O_rham	0.242	0.419	1.000									
DW_Quercetin	0.434	0.939	0.514	1.000								
DW_TA	0.224	0.477	0.347	0.453	1.000							
DW_T_acids	-0.040	0.001	0.375	0.081	0.674	1.000						
DW_Tot_sugars	0.037	0.570	0.401	0.632	0.915	0.503	1.000					
DW_fructose	-0.504	-0.537	-0.315	-0.555	0.231	0.021	0.177	1.000				
DW_glucose	0.547	0.167	-0.431	-0.084	0.383	0.274	0.077	0.014	1.000			
DW_sucrose	-0.318	0.264	0.660	0.522	-0.028	-0.039	0.302	-0.254	-0.875	1.000		
TP	0.316	0.369	0.604	0.468	-0.104	-0.208	-0.017	-0.359	-0.480	0.540	1.000	
TSS%	-0.718	-0.350	-0.284	-0.162	-0.174	-0.371	0.057	0.583	-0.543	0.330	0.116	1.000
	Q_gala	Q_glc	Q_rham	Quercetin	TA	T_acids	Tot_sugars	fructose	glucose	sucrose	TP	TSS%

D. 13(d): Correlation matrix for biochemical compounds of cv. Karutha Colomban peel ripened at 20°C and 30°C

%_Wt_loss	1.000						
DW_AsA	0.919	1.000					
DW_CA	0.910	0.841	1.000				
DW_K_3_O_glc	0.691	0.582	0.527	1.000			
DW_MA	0.487	0.601	0.684	-0.210	1.000		
DW_Mangi	-0.768	-0.919	-0.836	-0.467	-0.698	1.000	
DW_OA	-0.700	-0.423	-0.474	-0.801	0.215	0.143	1.000
DW_Q_3_O_gala	-0.223	-0.340	-0.585	0.145	-0.789	0.639	-0.392
DW_Q_3_O_glc	0.251	0.167	0.140	0.868	-0.557	-0.158	-0.556
DW_Q_3_O_rham	0.401	0.068	0.372	0.707	-0.351	0.030	-0.810
DW_Quercetin	-0.796	-0.835	-0.502	-0.690	-0.145	0.591	0.630
DW_TA	-0.646	-0.378	-0.395	-0.816	0.306	0.092	0.994
DW_T_acids	0.072	0.296	0.367	-0.454	0.872	-0.550	0.634
DW_Tot_sugars	-0.863	-0.860	-0.962	-0.621	-0.607	0.915	0.402
DW_fructose	-0.536	-0.595	-0.822	-0.193	-0.778	0.816	-0.056
DW_glucose	-0.801	-0.714	-0.823	-0.873	-0.205	0.727	0.630
DW_sucrose	-0.865	-0.892	-0.651	-0.458	-0.476	0.656	0.531
TP	0.068	-0.254	0.282	-0.113	0.142	0.235	-0.246
TSS%	-0.408	-0.396	-0.745	-0.155	-0.655	0.646	-0.051
	%_Wt_loss	DW_AsA	DW_CA	DW_K_3_O_glc	DW_MA	DW_Mangi	DW_OA

DW_Q_3_O_gala	1.000											
DW_Q_3_O_glc	0.236	1.000										
DW_Q_3_O_rham	0.163	0.686	1.000									
DW_Quercetin	-0.230	-0.333	-0.132	1.000								
DW_TA	-0.468	-0.606	-0.803	0.632	1.000							
DW_T_acids	-0.885	-0.577	-0.583	0.201	0.702	1.000						
DW_Tot_sugars	0.622	-0.309	-0.346	0.518	0.339	-0.376	1.000					
DW_fructose	0.934	0.004	-0.083	0.067	-0.135	-0.733	0.859	1.000				
DW_glucose	0.341	-0.675	-0.649	0.535	0.600	0.020	0.901	0.639	1.000			
DW_sucrose	-0.014	0.014	-0.005	0.915	0.496	-0.073	0.585	0.239	0.448	1.000		
TP	-0.252	-0.157	0.562	0.470	-0.182	0.002	-0.111	-0.252	-0.114	0.266	1.000	
TSS%	0.922	-0.042	-0.230	-0.138	-0.129	-0.647	0.772	0.963	0.610	0.024	-0.451	1.000
	Q_3_O_gala	O_glc	O_rham	Quercetin	TA	T_acids	Tot_sugars	fructose	glucose	sucrose	TP	TSS%

D. 14(a): Correlation matrix of cv. Willard ripened at 20°C and 30°C

%_wt_loss	1.000									
DW_K_3_O_glc	0.164	1.000								
DW_Mangi	0.629	0.430	1.000							
DW_Q_3_O_gala	-0.676	0.015	-0.219	1.000						
DW_Q_3_O_glc	-0.074	0.496	0.536	0.674	1.000					
DW_Q_3_O_rham	0.269	0.359	0.194	0.242	0.419	1.000				
DW_Quercetin	0.206	0.560	0.746	0.434	0.939	0.514	1.000			
TP	0.341	-0.148	0.207	0.316	0.369	0.604	0.468	1.000		
TSS%	0.794	-0.319	0.411	-0.718	-0.350	-0.284	-0.162	0.116	1.000	
Tot_caro	0.898	0.330	0.606	-0.448	0.110	0.385	0.387	0.556	0.574	1.000
	%_wt_loss	K_3_O_glc	Mangi	Q_3_O_gala	Q_3_O_glc	Q_3_O_rham	Quercetin	TP	TSS%	Tot_caro

D. 14(b): Correlation matrix of cv. K.Colomban ripened at 20°C and 30°C

%_Wt_loss	1.000									
DW_K_3_O_glc	0.691	1.000								
DW_Mangi	-0.768	-0.467	1.000							
DW_Q_3_O_gala	-0.223	0.145	0.639	1.000						
DW_Q_3_O_glc	0.251	0.868	-0.158	0.236	1.000					
DW_Q_3_O_rham	0.401	0.707	0.030	0.163	0.686	1.000				
DW_Quercetin	-0.796	-0.690	0.591	-0.230	-0.333	-0.132	1.000			
TP	0.068	-0.113	0.235	-0.252	-0.157	0.562	0.470	1.000		
TSS%	-0.408	-0.155	0.646	0.922	-0.042	-0.230	-0.138	-0.451	1.000	
T_Caro	0.828	0.884	-0.371	0.232	0.596	0.754	-0.740	0.141	-0.090	1.000
	%_Wt_loss	K_3_O_glc	Mangi	Q_3_O_gala	Q_3_O_glc	Q_3_O_rham	Quercetin	TP	TSS%	T_Caro

D. 15: Correlation matrix for aroma compounds of cvs. Willard, Karuhtha Colomban and Malgova

A_humulene	1.000									
A_pinene	0.817	1.000								
A_terpinene	-0.126	0.120	1.000							
B_caryophyllene	0.989	0.841	-0.107	1.000						
B_pinene	0.797	0.997	0.085	0.820	1.000					
Limonene	0.436	0.735	0.726	0.464	0.715	1.000				
Myrcene	0.842	0.938	-0.195	0.855	0.948	0.489	1.000			
Ocimene	0.761	0.460	-0.394	0.712	0.458	0.019	0.612	1.000		
Terpinolene	-0.185	0.058	0.958	-0.167	0.031	0.713	-0.252	-0.446	1.000	
car_3_ene	-0.073	0.021	0.261	-0.077	0.032	0.377	-0.084	-0.226	0.513	1.000
	A_humulene	A_pinene	A_terpinene	B_caryophy	B_pinene	Limonene	Myrcene	Ocimene	Terpinolene	car_3_ene